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(71) Applicant *(for all designated States except US)*: ILEX ONCOLOGY, INC. [US/US]; Suite 300, 11550 IH 10 West, San Antonio, TX 78230–1064 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: WEIS, Alexander [–/US]; 22 Galeria Drive, San Antonio, TX 78257 (US). SHAKED, Ze'ev [US/US]; Apartment #2, 52 Beacon Street, Boston, MA 02108 (US).

(74) Agent: MAYFIELD, Denise, L.; Vinson & Elkins, L.L.P., Suite 2300, 1001 Fannin Street, Houston, TX 77002–6760 (US).

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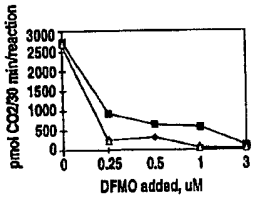
(57) Abstract

Preparations comprising a single enantiomer or defined non–racemic ratio of enantiomers of alpha–difluoromethylornithine (DFMO) are provided for treating, preventing, controlling the growth of and/or reducing the risk of cancers, tumors and other related neoplastic disorders. The preparations containing a substantially pure preparation of (–)–DFMO or a defined non–racemic ratio of DFMO enantiomers will provide an enhanced pharmacological activity relative to a preparation comprising racemic DFMO.



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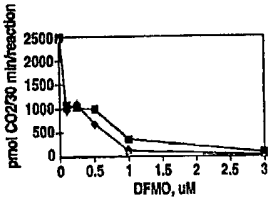

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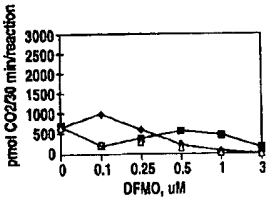

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ISOMERIC PHARMACEUTICAL FORMULATION CONTAINING DFMO FOR THE TREATMENT OF CANCER

This application claims the benefit of U.S. Provisional Application No. 60/033,070, filed December 13, 1996.

FIELD OF THE INVENTION

The present invention relates to the field of pharmaceutical formulations and methods for treating cancer. More specifically, the invention relates to compositions comprising a single or defined ratio of alpha-difluoromethylornithine enantiomer(s) having an improved pharmacological profile as compared to racemic DFMO for the treatment, prevention and/or controlling the growth rate of cancers, tumors and related disorders.

BACKGROUND OF THE INVENTION

ODC (ornithine decarboxylase) catalyzes the first step in the biosynthesis of putrescine (a diamine), spermidine and spermine, the three major polyamines of mammalian cells. *In vitro* studies show that polyamines participate in nearly all aspects of DNA, RNA, and protein synthesis. Polyamine accumulation is required to maintain maximum rates of cell proliferation. Blockage of polyamine accumulation by administration of DFMO and other inhibitors during accelerated cell growth results in a significant reduction of growth in a variety of cell systems. Since the only pathway to polyamine synthesis is via ornithine, synthesis depends on the activity of ODC. ODC is present in very small amounts in resting cells but can be increased many-fold within a few hours of exposure to hormones, drugs, and growth factors.

Specifically, DFMO causes a marked reduction of putrescine synthesis *in vivo*. As a result, putrescine and spermidine levels become undetectable, but there is little change in the concentration of spermine. Inhibition of polyamine synthesis must be maintained by continuous high levels of DFMO since ODC has a short half-life and is rapidly resynthesized.

Despite extensive evidence of the importance of polyamines for both normal and tumor cell growth, the precise role polyamines play in regulation of cellular proliferation is largely unknown. It is thought that these compounds, which are highly basic cations with a

high affinity for nucleic acids, are involved in nucleic acid mediated regulation of cellular proliferation and secretory activity (Danzin et al., 1979a; Danzin et al., 1979b; McKeehan et al., 1982). It has also been demonstrated that polyamine depletion induced by DFMO adversely affects the cellular DNA synthetic machinery by reducing the rate of DNA elongation (Oredsson, Nicander and Heby, 1990).

Regulation of certain oncogenes has been shown to be important in cellular growth. More specifically, regulation of the N-*myc* may be important because its amplification is associated with aggressive malignant behavior in neuroblastomas. *In vitro* and *in vivo*, DFMO has been shown to decrease polyamine levels, inhibit cellular growth and decrease N-*myc* mRNA levels in neuroblastoma cells. These data suggest that polyamine depletion plays a role in the regulation of N-*myc* expression at the level of transcription and may therefore affect cellular growth via N-*myc* gene expression (Iyer and Franz, 1990). Additionally, in human colon carcinoma cells, DFMO-induced polyamine depletion has also resulted in greater than a 90% decrease in the mRNA expression of c-*myc* (Celano et al., 1988).

The pharmacology of racemic preparations of DFMO alone have been previously studied. For example, in a small clinical study of patients (12 with leukemia, 2 with multiple myeloma) received racemic DFMO 4-32 g/m^2 IV or orally (PO). Seven to 10 days were required before DFMO was detected (>1 μM) in the plasma or in mononuclear cells when racemic DFMO was administered by the PO route. With continuous IV infusion (CI), this level (>1 μM) was established within 3 days. Decreases in mononuclear cell polyamine concentrations were not achieved until the cellular DFMO concentration exceeded 2-5 μM. Putrescine levels decreased in six patients while spermidine decreased in only two patients. Inhibition of polyamine synthesis was associated with either stabilization or a decrease in leukemia blasts (Maddox et al., 1984).

Racemic DFMO has been reported to inhibit cellular replication *in vitro* in several malignant animal tumor cell lines, specifically in L1210 and L5178Y leukemia, rat hepatoma, mouse mammary sarcoma (EMT6) and hamster pancreatic adenocarcinoma (H2T) cell lines (Mamont et al., 1978; Prakash et al., 1980; Pera et al., 1986; Marx et al., 1987).

Racemic DFMO *in vitro* has also been reported to have growth inhibitory effects in certain human carcinoma cell lines. Specifically, eight different small cell lung cancer (SCLC) cell lines treated with DFMO (5 mM) during the exponential growth phase were reported to have an initial inhibition of cell growth followed by a progressive and complete

loss of cells from the cultures. In contrast to SCLC, four lines of human non-small cell lung cancer (two adenocarcinoma, one squamous cell carcinoma, and one large cell undifferentiated carcinoma) were reported to show no cell loss over treatment periods of up to 8 weeks despite a cessation of cell proliferation during DFMO treatment (Luk et al., 1982). DFMO has also been reported to inhibit human HeLa cell growth *in vitro* by causing a decrease in the intracellular levels of putrescine and spermidine (Sunkara et al., 1980). In a clonogenic assay, the inhibitory effects of DFMO against two human pancreatic tumor cell lines (PANC-1 and COLO 357) were reported to be predominantly cytostatic, reversible by putrescine, and additive when combined with cisplatin (Chang et al., 1984). When Neelam et al. (1990) combined racemic DFMO with flavone acetic acid against human colon cancer cells, reported results for the combination were not significantly different from those of DFMO alone.

The anti-tumor action of DFMO *in vivo* was assessed using the following malignant animal cell lines and routes of administration: L1210, intraperitoneal inoculation (IP) (Prakash et al., 1978); Lewis lung (LL) carcinoma, intramuscular inoculation (IM) (Bartholeyns, 1983); B16 murine melanoma, subcutaneous inoculation (SC) (Sunkara and Rosenberger, 1987); hamster EMT6 sarcoma, SC (Prakash et al., 1980); and M3 murine adenocarcinoma, SC (Klein et al., 1985). All malignant cell lines were inoculated into mice. Following exposure of the mice to DFMO, the growth of all tumors was significantly reduced (Prakash et al., 1978; Bartholeyns 1983; Sunkara and Rosenberger, 1987; Klein et al., 1985). The detectable metastases were also reportedly reduced in animals receiving the B16 melanoma, M3 adenocarcinoma and LL carcinoma cell lines (Bartholeyns, 1983; Sunkara and Rosenberger, 1987; Klein et al., 1985). DFMO treatment in animals with H2T-cell tumors inhibited growth of these pancreatic cancer cells by as much as 50% of control (Marx et al., 1987).

The effect of DFMO *in vivo* with human cells was examined by implanting a small cell lung carcinoma cell line into athymic mice. DFMO administered to these mice markedly inhibited tumor growth, as well as increased mean animal survival (Luk et al., 1983). The growth of six other human tumors (three mammary carcinomas, a malignant melanoma, a bladder carcinoma, and an endocervical carcinoma), was significantly decreased after DFMO treatment compared to growth in control mice.

The chemopreventive effects of DFMO (given as a dietary supplement) were assessed in three rodent models of human epithelial cancer (Ratko et al., 1990). DFMO provided

significant protection against 7,12-dimethylbenz(a) anthracene (DMBA)-induced mammary carcinogenesis in rats. In mice, the incidence of N-butyl-N(4-hydroxybutyl) nitrosamine (OH-BBN)-induced bladder cancer was reduced by 54-62% versus the control group when DFMO was given prior to the administration of the first dose of OH-BBN. Furthermore, in OH-BBN treated mice consuming a high dose of DFMO, the lesions which did occur tended to be less invasive than those observed in mice receiving either DFMO at a low dose or the basal diet alone (no DFMO). In hamsters, continuous treatment with DFMO reduced the incidence and size of tracheal carcinomas occurring in animals exposed to the carcinogen methylnitrosourea.

The cutaneous injection (CI) of DFMO (6-14 g/m^2 for 28 days) in patients with metastatic colorectal cancer was studied (Ajani et al., 1988). The plasma DFMO levels were maintained between 400 and 600 μM. No complete or partial responses were observed. However, three patients showed minor responses lasting less than three months. The predominant hematological toxicity was thrombocytopenia although some degree of granulocytopenia and anemia also occurred. Non-hematological toxic effects included reversible hearing loss (8/33), malaise (17/33) and nausea, vomiting and diarrhea (8/33).

A Phase I study of hepatic artery infusion of DFMO in doses ranging from 0.5 to 2.0 g/m^2/day by CI to patients with metastatic liver disease has also been reported (Lipton et al., 1987). The dose limiting toxicity (DLT) with this route of administration was tinnitus. Reversible loss of high frequency hearing occurred at doses greater than or equal to 1.0 g/m^2/d.

DFMO and alpha IFN has also been examined in cancer patients. (Edmonson et al., 1988). In one study, patients received alpha-IFN from 0.4-6.4 MU/m^2 IM daily for 14 days and DFMO from 1.5-2.5 g/m^2 p.o. every 6 hours for 14 days. Some patients with metastatic malignant melanoma were reported to have partial responses while gastrointestinal toxicity (nausea, vomiting, diarrhea) were the DLT. The recommended Phase II dose was 3.2 MU/m^2/d alpha-IFN and 1.5 g/m^2 DFMO/every 6 hours (Talpaz et al., 1986).

The combination of alpha interferon (IFN) and DFMO in the treatment of patients with metastatic melanoma has also been examined (Croghan et al., 1988). Patients were given DFMO p.o. at a dose of 4 or 6 g/m^2/d for 11 days in combination with an intramuscular injection of 1.5, 3.0, 6.0 or 9.0 MU/m^2 of alpha-IFN. The MTD was 4 g/m^2/d of DFMO plus 6 MU/m^2 of alpha IFN. The DLT consisted of leukopenia, fatigue and weight loss. Other toxicities included reversible hearing loss, diarrhea, nausea, and vomiting. Responses seen

in some patients included a partial response of soft tissue metastases, a partial response of lung and liver metastases, and a complete regression of liver metastases without clearance of carcinomatous meningitis.

The combination of DFMO and MGBG in refractory lymphoblastic and myeloblastic childhood leukemia has also been examined. (Siimes et al., 1981). These patients were not responding to conventional cytostatic therapy and were considered to be in their terminal relapse. Treatment consisted of sequential dosing of MGBG (500-700 mg/m^2), given first as a single dose over 3 hours, followed by DFMO (3-15 g/m^2) given for 3-5 days. It was determined that administering DFMO first greatly increased cellular uptake of subsequently administered MGBG. Toxicities associated with the combined drug regimen were mild (transient fever, decrease in platelet count and gastrointestinal upset).

Work has been done to determine the dose and duration of DFMO pre-treatment necessary to deplete human polyamine levels and to increase tumor cellular uptake of MGBG. In one study, patients with hematological malignancies were treated with DFMO doses ranging from 4-8 g/m^2 PO/8 hours for 7 days, followed by an MGBG dose of 500 mg/m^2 IV weekly (Maddox et al., 1988). The number of circulating blast cells decreased in all patients treated for more than one week with the DFMO/MGBG combination. No correlation between circulating white blood cell number, circulating tumor cell doubling time, or percent of tumor cells in S-phase to polyamine levels was observed. Toxicities observed included myalgias, diarrhea, GI upset, mucositis, loss of hearing, thrombocytopenia, and ascending paralysis.

A combination of 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) and DFMO has also been evaluated in the treatment of primary recurrent gliomas and glioblastomas (Prados et al., 1989). Toxicity, time to tumor progression as measured from initiation of therapy, and objective response rates in both adults and children with recurrent brain tumors was evaluated. Moderate toxicities at these doses were reported with at least some incidence of progressive hearing loss and severe myelosuppression.

A clinical study to determine the pharmacokinetics of racemic DFMO in healthy men has been reported. (Haegele, 1981). Racemic DFMO possesses a short elimination half-life, i.e. $t_{1/2}$ about 3.5 hours, as it undergoes rapid renal elimination. DFMO is not highly protein bound nor does it undergo extensive metabolism prior to elimination. After oral administration of racemic DFMO containing solutions, peak plasma concentrations occur within about 6 hours. The decay of the plasma concentrations followed first-order kinetics.

The mean total body clearance is about 1.20 mL/min/Kg. The mean renal clearance is about 0.99 mL/min/Kg accounting for 83% of drug elimination. The mean apparent volume distribution is about 0.337 L/Kg corresponding to 24L for a 70 Kg man. The amount of unchanged drug in 24-hour urine samples is about 44% after oral administration and about 80% after I.V. administration.

The maximally tolerated dose (MTD) of oral DFMO has also been examined (Abeloff et al., 1984). The MTD of a 4 day DFMO course given orally, by CI, or by pulse IV infusions (Griffin et al., 1987) to patients with advanced solid tumors or lymphomas has also been studied. Some patients receiving twenty-four courses of oral DFMO on a 28 day schedule developed thrombocytopenia (the DLT). Gastrointestinal side effects have also been observed in treated patients (Abeloff et al., 1984). Audiometric abnormalities is a further side effect associated with DFMO treatment (Griffin et al., 1987). No therapeutic responses were noted in these patient populations.

A study by Griffin et al. (1987) compared routes (PO, CI and IV) and schedules (bolus and continuous infusions) of administration. Nausea and vomiting were the most frequent and severe toxicities noted, but this occurred mainly in patients receiving oral DFMO, with diarrhea occurring in patients receiving oral DFMO. Mild leukopenia was observed with all routes of administration. Thrombocytopenia occurred in only two patients and was mild. No audiometric abnormalities were reported on the 4 day schedule. No therapeutic responses were reported with any route of drug administration. The MTD of the oral DFMO on the 4 day schedule was 3.75 g/m^2 every 6 hours. No MTD was reached with either type of IV administration.

The known minimum effective dose (MED) for DFMO in significantly reducing polyamine pools *in vivo* is about 0.43 g/day. The maximum tolerated dose is about 12 g/m^2/day by chronic oral administration. The known minimum toxic dose for DFMO, in terms of ototoxicity, is about 150 g/m^2 cumulative dose based upon 0.25-6.0 g/m^2/day chronic oral administration.

At a dose of 3 g/m^2, a steady state level of DFMO, 386-622, μM was achieved. This level is within the range needed for ODC inhibition in cell-culture systems, as well as for the inhibitory activity against various human tumors *in vitro*. A DFMO dose of 2.25 g/m^2 every six hours was recommended for Phase II studies in patients previously treated with cytotoxic drugs (Abeloff et al., 1984).

Racemic DFMO associated ototoxicity generally begins to occur once a cumulative dose of about 150 g/m^2 has been received. The ototoxicity is exhibited by loss of hearing acuity at the upper end of the frequency scale and is completely reversible upon discontinuation of DFMO therapy. GI toxicity occurs predominantly during P.O. rather than I.V. administration of DFMO.

Although drugs possessing a pharmacokinetic profile such as that for DFMO are generally poor candidates for sustained release formulations, some are known for racemic DFMO. Tricalcium phosphate (TCP) and aluminum calcium phosphate (AlCAP) capsule formulations have been tested as implants in rats and proposed for the treatment of trypanosomiasis. There has been no teaching of a sustained release oral formulation for DFMO for the treatment of cancer related disorders.

A layered tablet formulation comprising racemic DFMO and a slow release layer compressed to a rapid release layer has been tested for controlling fertility and gestation in rat and mouse models. (Bey et al., U.S. 4,309,442). Conventional release hard gelatin capsule and tablet formulations comprising racemic DFMO are also known and have been tested in rat, dog and/or mouse models for controlling gestation, treating non-malignant proliferative skin diseases and/or cancer chemoprevention. (Bey et al., U.S. 4,496,588).

It is known in the pharmaceutical industry that the individual enantiomers of chiral compounds may possess different pharmacological profiles i.e., differences in pharmacokinetics, toxicities, pharmacologic activities, efficacy, etc. (-)-DFMO has been eported to be the enantiomer primarily responsible for ODC inhibition. However, the side effects associated with DFMO have not been traced to a particular enantiomeric form of this agent.

Individual enantiomers of DFMO display different pharmacokinetic profiles after continuous infusion in cancer patients and after I.V. or oral administration in sleeping sickness patients. (Schmitt-Hoffman AH, Haegele KD, 1987) (-)-DFMO represents 45 ± 5% (mean + SD, n=41) of the total DFMO in the plasma and 47 ± 1.8% (n=77) of the total DFMO in the urine of cancer patients receiving a continuous infusion of racemic DFMO. In Sleeping Sickness patients receiving racemic DFMO, (-)-DFMO represents 48 ± 0.4% (n=17) and 35 ± 2.8% (n=12) of the total DFMO in the plasma after I.V. and oral administration, respectively. However, it is not known whether the (+)- or (-)-DFMO enantiomer is primarily responsible for the toxicological profile attributed to racemic DFMO.

Given the myriad of what are sometimes severe side effects associated with conventional racemic DFMO treatment regimens used, a need continues to exist in the medical arts for improved formulations having an enhanced pharmacological profile for the treatment, prevention and control of cancers and tumors.

It is an object of the present invention to provide a pharmaceutical composition for the treatment or prophylaxis of cancer, the composition having improved pharmacological activity relative to racemic DFMO with reduced toxicity.

It is another object of the invention to provide a method of treating, preventing, controlling the growth of and/or reducing the risk of cancer by administering to a patient a pharmaceutical composition as described above.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a pharmaceutical composition having enhanced pharmacological activity relative to racemic DFMO for treating, preventing, controlling the growth of and/or reducing the risk of cancer, tumors and related neoplastic disorders. In some embodiments, these compositions comprise a therapeutic amount of L-DFMO or a defined non-racemic ratio of (+)-DFMO: (-)-DFMO or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier. More specifically, the composition may comprise substantially optically pure (-)-DFMO or a defined non-racemic ratio of (-)-DFMO: (+)-DFMO, and will have reduced side effects or toxicity, enhanced therapeutic efficacy and/or improved pharmacokinetics relative to racemic DFMO.

It is contemplated and within the scope of the invention that a composition as described above will be useful for the inhibition of malignant cellular proliferation and/or the amelioration of a wide variety of cancers, tumors and related neoplastic disorders.

It is also contemplated and within the scope of the invention that the pharmaceutical composition can comprise an optically pure enantiomer or a defined ratio of the (+):(-) enantiomers of DFMO in combination with other therapeutic compounds or cytotoxic agents for the treatment or prophylaxis of cancer, as well as for the inhibition of cancer and tumor growth rate.

Another aspect of the present invention provides a medicament for use in treating, preventing, controlling the growth of and/or reducing the risk of cancer, tumors and related neoplastic disorders in a patient in need of such treatment. In some embodiments, this

medicament a therapeutically effective amount of pharmaceutical composition comprising (-)-DFMO or a defined non-racemic ratio of (+)-DFMO: (-)-DFMO, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier, the composition providing enhanced pharmacological activity relative to racemic DFMO.

In some embodiments, the pharmaceutical preparations and medicaments of the present invention in the non-racemic formulations comprise a defined ratio of (+)-DFMO:(-)-DFMO of about 5:95 to about 45:55 by weight, respectively. In others, the ratio of (+)-DFMO to the (-)-DFMO is about 10:90, or 20:80, or 25:75, by weight, respectively.

Other features, advantages and embodiments of the invention will be apparent to those skilled in the art from the following description, accompanying examples and appended claims.

DETAILED DESCRIPTION OF THE DRAWINGS

FIG 1A - FIG 1C. FIG 1A - ODC activity vs DFMO concentration, ornithine concentration 200 μM (-♦- = DL-DFMO; -■- = D-DFMO, L-DFMO). FIG 1B - ODC activity vs DFMO concentration, ornithine concentration 100 μM (-♦- = DL-DFMO; -■- = D-DFMO, L-DFMO). FIG 1C - ODC activity vs DFFMO concentration, ornithine concentration 25 μM (-♦- = DL-DFMO; -■- = D-DFMO, L-DFMO).

FIG 2A - FIG 2C. FIG 2A - ODC activity vs DFMO concentration, ornithine concentration 200 μM (-♦- = DL-DFMO; -■- = D-DFMO, L-DFMO). FIG 2B - ODC activity vs DFMO concentration, ornithine concentration 100 μM (-♦- = DL-DFMO; -■- = D-DFMO, L-DFMO). FIG 2C - ODC activity vs DFMO concentration, ornithine 25 μM (-♦- = DL-DFMO; -■- = D-DFMO, L-DFMO).

FIG 3A - FIG 3C. FIG3A - DL-DFMO (♦ 1/control; ■ 1/DL-0.1, 1/DL-0.25; X 1/DL-0.50; χ 1/DL-1; ●1/DL-3; —Linear (1/DL-3); —Linear (1/DL-1); —Linear (1/DL-0.50); —Linear (1/DL-0.1); —Linear (1/DL-0.25)). FIG 3B - D-DFMO (♦ 1/control; ■ 1/D-0.1, 1/D-0.25; X 1/D-0.5; χ 1/D-1; ●1/D-3;—Linear (1/D-1); —Linear (1/control); —Linear (1/D-0.1); —Linear (1/D-0.25; —Linear (1D-3)). FIG 3C - L-DFMO (♦ 1/control; ■ L-0.1, L-0.25; X L-0.5; χ L-1; —Linear (1/control); —(L-0.1); —Linear (L-0.25); —Linear (L-0.5) —Linear (L-1)).

FIG 4A - FIG 4C demonstrate the effect of 24 hour treatment of different concentrations of DFMO mixtures on HCT116 cells. FIG 4A demonstrates the effect of

racemic DFMO mixture on HCT116 cells. FIG 4B demonstrates the effect of D-DFMO on HCT116 cells (—■— = D - 0.1 mM; —□— = D - 0.5 mM; —♦— = D - 1.0 mM; —◇— = D - 5.0 mM; —▲— = Control). FIG 4C demonstrates the effect of L-DFMO on HCT 116 cells (—■— = L - 0.1 mM; —□— = L - 0.5 mM; —♦— = L - 1.0 mM; —◇— = L - 5.0 mM; —▲— = Control).

FIG 5 demonstrates the speed of depletion in HCT116 cells after 24 hour treatment with DFMO.

FIG 6A - FIG 6E. High Pressure Liquid Chromatography Analysis of D-DFMO. FIG 6A- HPLC Analysis of Control (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 6B - HPLC Analysis of D-DFMO at 0.1 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 6C - HPLC Analysis of D-DFMO at 0.5 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 6D - HPLC Analysis of D-DFMO at 1.0 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 6E - HPLC Analysis of D-DFMO at 5.0 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine).

FIG 7A - FIG 7D. High Pressure Liquid Chromatography Analysis of L-DFMO. FIG 7A - HPLC Analysis of L-DFMO at 0.1 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 7B - HPLC Analysis of L-DFMO at 0.5 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 7C - HPLC Analysis of L-DFMO at 1.0 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 7D - HPLC Analysis of L-DFMO at 5.0 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine).

FIG 8A - FIG 8D. High Pressure Liquid Chromatography Analysis of Racemic (R) DFMO. FIG 8A - HPLC Analysis of R-DFMO at 0.1 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 8B - HPLC Analysis of R-DFMO at 0.5 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 8C - HPLC Analysis of R-DFMO at 1.0 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine). FIG 8D - HPLC Analysis of R-DFMOat 5.0 mM (-◇- = Putrescine; -□- = Spermidine; -△- = Spermine).

DETAILED DESCRIPTION OF THE INVENTION

Pharmaceutical Composition Comprising (-)-DFMO

In one aspect, the invention provides a pharmaceutical composition having enhanced pharmacological activity as compared to racemic DFMO, the composition comprising a substantially pure preparation of (-)-alpha-difluoromethylornithine ((-)-DFMO or

(L)-DFMO). As used in the description of the present invention, a "substantially pure preparation" of a particular enantiomeric from DFMO is defined as a preparation comprising no less than 95 % wt. of a first DFMO enantiomer and no more than 5% wt. of a second DFMO enantiomer, of the D- or L- enantiomer, respectively.

As used herein, the term "enhanced pharmacological activity" means improved bioavailability, reduced toxicity or side effects, enhanced therapeutic or clinical benefit and/or enhanced pharmacological profile.

It is known that the k_i for (-)-DFMO for ODC inhibition is at least ten fold greater than that for (+)-DFMO. Thus, the ODC inhibition observed *in vivo* following administration of racemic DFMO is largely (approximately 90%) due to (-)-DFMO rather than (+)-DFMO. Therefore, a composition comprising optically pure (-)-DFMO will provide a more convenient delivery of the drug by advantageously requiring less active drug substance, on a mole basis, to yield the same or better therapeutic effect achieved with racemic DFMO.

Since (+)-DFMO binds to ODC, it is able to compete with (-)-DFMO for the ODC binding site. Thus, in the absence of (+)-DFMO, a substantially pure preparation of (-)-DFMO will have improved ODC inhibitory activity which can translate into improved bioavailability, pharmacokinetics, pharmacology and therapeutic benefit.

Since ototoxicity is associated with the total cumulative dose of racemic DFMO received by chronic administration, it is likely that residual accumulation of one of the enantiomers of DFMO or its metabolites is also ototoxic. The pharmacokinetic data of the enantiomers has shown that the concentration of (-)-DFMO in plasma is lower than that for (+)-DFMO. It has also been reported that only about 83% of total DFMO can be accounted for after clearance from the body of racemic DFMO. Thus, 17% of any given dose is unaccounted for and will likely lead to residual accumulation of DFMO. Since (-)-DFMO is present at a lower plasma concentration level than (+)-DFMO, it is less likely to accumulate upon chronic administration. Thus, a pharmaceutical composition comprising optically pure (-)-DFMO will reasonably exhibit less ototoxicity than one comprising racemic DFMO.

Pharmaceutical Composition Comprising a Defined Non-racemic Ratio of (+)-DFMO: (-)-DFMO

In another aspect, the invention provides a pharmaceutical composition having enhanced pharmacological activity as compared to racemic DFMO comprising a defined non-

racemic ratio of (+)-DFMO/(-)[-]DFMO. For a patient suffering from a high degree of hearing loss or tinnitus due to racemic DFMO therapy, the method of the present invention suggests administering a non-racemic DFMO-containing composition comprising 55%-95%/45%-5% wt. (-)-DFMO/(+)-DFMO, respectively, to provide a therapeutic benefit while reducing the observed ototoxicity.

For a patient requiring excessively high doses of racemic DFMO in order to obtain a therapeutic benefit, the present invention suggests administering a non-racemic DFMO-containing composition comprising 55%-95%/45%-5% wt. (-)-DFMO/(+)-DFMO, respectively, to provide an enhanced therapeutic benefit at a lower dose than is required with racemic DFMO.

Treating, Preventing, Controlling the Growth of and/or Reducing the Risk of Cancer, Tumors and Related Neoplastic Disorders With DFMO Enantiomers

As indicated above, racemic DFMO has found utility in a variety of neoplastic disorders such as neuroblastoma, colon carcinoma, leukemia, hepatoma, mammary sarcoma, small cell lung cancer, pancreatic tumor, Lewis lung carcinoma, B16 murine melanoma, M3 murine adenocarcinoma, bladder carcinoma, endocervical carcinoma, epithelial cancer, chemically induced cancer, metastatic colorectal cancer, refractory childhood leukemia, cervical intraepithelial neoplasia grade 3 (CIN III), hematological malignancies, acute and chronic myeloid leukemia, recurrent glioma and glioblastoma, solid tumor, lymphoma, mammary carcinoma, oral leukoplakia, premalignant polyps, tamoxifen resistant breast cancer, estrogen independent breast cancer, and Barrett's esophagus.

In each of the above indications, racemic DFMO has provided minor to partial to complete prevention and/or regression of or slowed the growth of the indicated new neoplastic disorder. It is believed that administration of a single enantiomer or a defined non-racemic ratio of enantiomers of DFMO to treat, prevent, reduce the risk or slow the growth of cancers and tumors will provide an enhanced pharmacological activity in a patient as compared to administration of racemic DFMO. Such an enhanced pharmacological activity can be demonstrated by employing methods described or incorporated by reference herein. When employing the methods incorporated herein by reference, (-)-DFMO or a defined non-racemic ratio of (+)-DFMO/(-)-DFMO will be used in place of the racemic DFMO referred to therein.

The effect of DFMO for the control of the growth of rapidly proliferating tumor or cancer tissue can be assessed in standard animal tumor models. For example, the anti-tumor effect of DFMO has been demonstrated in the following animal tumor models: (a) L1210 leukemia in mice, (b) EMT6 tumor in Balb/C mice, (c) 7,12-dimethylbenzanthracene-induced (DMBA-induced) mammary tumor in rats, and (d) Morris 7288C or 5123 hepatoma in Buffalo rats. In addition, the anti-tumor effect of DFMO in combination with various cytotoxic agents has been demonstrated as follows: (a) in combination with vindesine or adriamycin in L1210 leukemia in mice, in Morris 7288C hepatoma in Buffalo rats, and in EMT6 tumor in mice, (b) in combination with cytosine arabinoside in L1210 leukemia in mice, (c) in combination with methotrexate in L1210 leukemia in mice, (d) in combination with cyclophosphamide in EMT6 tumor in mice and in DMBA-induced tumor in mice, (e) in combination with BCNU in mouse glioma 26 brain tumor, and (f) in combination with MGBG in L1210 leukemia in mice, in Morris 7288C hepatoma in Buffalo rats, in P388 lymphocytic leukemia in mice, and in S-180 sarcoma in mice.

As used herein, the term "tumor" means both benign and malignant tumors or neoplasms, and includes melanomas, lymphomas, leukemias, and sarcomas. Illustrative examples of tumor tissues are cutaneous tumors, such as malignant melanomas and mycosis fungoides; hematologic tumors such as leukemias, for example, acute lymphoblastic, acute myelocytic or chronic myelocytic leukemia; lymphomas, such as Hodgkin's disease or malignant lymphoma; gynecologic tumors, such as ovarian and uterine tumors; urologic tumors, such as those of the prostate, bladder or testis; soft tissue sarcomas, osseus or non-osseus sarcomas, breast tumors; tumors of the pituitary, thyroid and adrenal cortex; gastrointestinal tumors, such as those of the esophagus, stomach, intestine and colon; pancreatic and hepatic tumors; laryngeae papillometastases and lung tumors.

The term "controlling the growth", as used herein, means slowing, interrupting, arresting, or stopping the growth and metastases of a rapidly proliferating tumor in a warm blooded animal. It should be understood that treatment (controlling the growth of a tumor tissue) in a warm blooded animal with (-)-DFMO, either with or without the added effects of an additional cytotoxic agent or therapeutic drug provides a clinical benefit; although, the tumor tissue need not be destroyed or totally eliminated. Experimentally, however, some tumor tissues have been completely eliminated. The formulations and methods disclosed herein can provide improved survivability and/or quality of life.

As used herein, the term "reducing the probability of" means reducing the incidence, or rate of occurrence or reoccurrence, of a given disorder in a patient receiving DFMO therapy. As used herein, the term "preventing" means eliminating the incidence of a given disorder in a patient receiving DFMO therapy.

As used herein, the term "patient" is taken to mean animals such as mammals, for example, dogs, rats, mice, cats, guinea pigs, horses, cows, sheep and humans.

When, in the treatment of a malignant neoplastic disease, DFMO is administered in combination with a cytotoxic agent, the therapeutic effect of the cytotoxic agent can be potentiated. The remission produced by the cytotoxic agent can be enhanced and regrowth of the tumor or cancer tissue can be slowed or prevented. DFMO can produce an additive or synergistic effect with a cytotoxic agent against a particular tumor. In combination with DFMO, the cytotoxic agent may, therefore, be administered at a lower dosage level or at less frequent intervals as compared to the cytotoxic agent when used alone. Thus, the detrimental and/or debilitating side effects of the cytotoxic agent are minimized while, at the same time, the anti-tumor effects are enhanced.

The term "combination therapy" contemplates the administration of non-racemic preparations of DFMO immediately prior to the beginning of therapy with a cytotoxic and/or cytostatic agent, concomitantly with such therapy, or during the period of time immediately following cessation of such therapy. Preferably, the patient is treated with DFMO for about 1 to 14 days, preferably 4 to 14 days, prior to the beginning of therapy with a cytotoxic agent, and thereafter, on a daily basis during the course of such therapy. Daily treatment with DFMO can be continued for a period of, for example, 1 to 365 days after the last dose of the cytotoxic agent is administered.

When such combination therapy results in remission of the tumor or cancer, and all tumor or cancer cells are not destroyed, regrowth of the tumor or cancer may be prevented or slowed indefinitely by continued treatment with DFMO.

As used herein, the term "therapeutic compound" is taken to mean a compound having the desired beneficial pharmacologic and therapeutic effects in an animal. Advantageously, the therapeutic compound is a cytotoxic agent and is also indicated for the treatment or prophylaxis of cancer and/or tumors.

The therapeutic compounds contemplated within the scope of the invention may be in their free acid, free base, or pharmaceutically acceptable salt forms. They may be derivatives or prodrugs of a given compound.

Loading of the therapeutic compounds into a pharmaceutical formulation may be accomplished following well known techniques such as those described in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, the disclosure of which is hereby incorporated by reference.

Therapeutic compound loading into the formulation may need to be varied according to the pharmacological activity of the compound, the indication being treated, the targeted dosing regimen, the projected method of administration, the integrity or stability of the final formulation or other such reasons.

For purposes of this invention, non-racemic preparations of DFMO can be administered to a patient in conjunction with other therapeutical methods. For example, DFMO can be administered in conjunction with surgical excision of the tumor or with radiation therapy, immunotherapy, or local heat therapy.

Illustrative examples of cytotoxic and/or cytostatic agents or therapeutic compounds which can be administered in combination with DFMO include, by way of example and without limitation:

Alkylating agents;

Alkyl Sulfonates such as Busulfan, Improsulfan, Piposulfan;

Aziridines such as Benzodopa, Carboquone, Meturedopa, Uredopa;

Ethylenimines and Methylmelamines such as Altretamine, Triethylenemelamine, Triethylenephosphoramide, Triethylenethiophosphoramide, Trimethylolomelamine;

Nitrogen Mustards such as Chlorambucil, Chlornaphazine, Cholophosphamide, Estramustine, Ifosfamide, Mechlorethamine, Mechlorethamine Oxide Hydrochloride, Melphalan, Novembichin, Phenesterine, prednimustine, Trofosfamide, Uracil Mustard;

Nitrosoureas such as Carmustine, Chlorozotocin, Fotemustine, Lomustine, Nimustine, Ranimustine;

Antibiotics such as Aclacinomycins, Actinomycin F_1, Authramycin, Azaserine, Bleomycins, Cactinomycin, Carubicin, Carzinophilin, Chromomycins, Dactinomycin, Daunorubicin, 6-Diazo-5-oxo-L-norleucine, Doxorubicin, Epirubicin, Mitomycins, Mycophenolic Acid, Nogalamycin, Olivomycins, Peplomycin, potfiromycin, Puromycin, Streptonigrin, Streptozocin, Tubercidin, Ubenimex, Zinostatin, Zorubicin;

Antimetabolites;

Folic Acid Analogs such as Denopterin, Methotrexate, Pteropterin, Trimetrexate;

Purine Analogs such as Fludarabine, 6-Mercaptopurine, Thiamiprine, Thioguanaine;

Pyrimidine Analogs such as Ancitabine, Azacitidine, 6-Azauridine, Carmofur, Cytarabine, Dideoxyuridines, Doxifluridine, Enocitabine, Floxuridine, Fluororacil, Tegafur;

Others such as Aceglatone, Aldophosphamide Glycoside, Aminolevulinic Acid, Amsacrine, Bestrabucil, Bisantrene, Carboplatin, Cisplatin, Defofamide, Demecolcine, Diaziquone, Elliptinium Acetate, Etoglucid, Etoposide, Gallium Nitrate, Hydroxyurea, Interferon-α, Interferon-β, Interferon-γ, Interleukin-2, Lentinan, Lonidamine, Mitoguazone, Mitoxantrone, Mopidamol, Nitracrine, Pentostatin, Phenamet, Pirarubicin, podophyllinice Acid, 2-Ethylhydrazide, Procarbazine, PSK®, Razoxane, Sizofiran, Spirogermanium, Tamoxifen, Taxol, Teniposide, Tenuazonic Acid, Triaziquone, 2,2'2" -Trichlorotriethylamine, Urethan, Vinblastine, Vincristine, Vindesine, Dacarbazine, Mannomustine, Mitobronitol, Mitolactol, Tamoxifen and Pipobroman;

Androgens such as Calusterone, Dromostanolone Propionate, Epitiostanol, Mepitiostane, Testolactone;

Antiadrenals such as Aminoglutethimide, Mitotane, Trilostane;

Antiandrogens such as Flutamide, Nilutamide;

Antiestrogens such as Aromatase Inhibiting 4(5)-Imidazoles; and

Folic Acid Replenisher such as Frolinic Acid.

The therapeutic compound(s) contained within the formulation may be formulated as their pharmaceutically acceptable salts. As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent pharmacologically active compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfonic, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as amino acids, acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent pharmacologically active compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a predetermined amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two. Generally, nonaqueous media are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

It should be understood that methods for the treatment of the above-mentioned disorders will employ a variety of dosage forms and dosing regimens, i.e., each indication will have associated with it an optimal dosage form and dosing regimen.

Dosage Form

The pharmaceutical composition of the present invention can be administered by a variety of routes such as, by way of example and without limitation: intraperitoneal, intra-articular, intra-arterial, intracardiac, intracavity, intracartilaginous, intradermal, intrathecal, intraocular, intraspinal, intrasynovial, intrathoracic, intratracheal, intrauterine, epidural, percutaneous, intravascular, intravenous, intracoronary, intramuscular or subcutaneous injection; inhalation; or oral, nasal, buccal, rectal, ophthalmic, otic, urethral, vaginal, or sublingual administration. Such methods of administration and others contemplated within the scope of the present invention are known to the skilled artisan.

The present pharmaceutical composition can be provided in a variety of dosage forms such as, by way of example and without limitation, solution, suspension, cream, ointment, lotion, capsule, tablet, caplet, gelcap, suppository, enema, transdermal patch, implant, gel, injectable, i.v. infusion bag or bottle, aerosol, concentrate, dressing, elixir, syrup, emulsion, film, granule, gum, insert, intrauterine device, jelly, form, paste, pastille, pellet, shampoo, sponge, spray, swab, tampon, tape, troche, lozenge, dental cone, diaphragm, disk, douche,

inhalant, magma, mouthwash, ophthalmic continuous release core unit, poultice, stick, strips, toothpaste, tooth powder and wafer.

Methods for the preparation of the dosage forms contemplated herein are described in the included examples or in the cited references, the disclosures of which are hereby incorporated herein in their entirety. Any ingredients used in the present formulation should not degrade or decompose a significant portion of the DFMO or other therapeutic compound(s) used prior to administration.

The term "unit dosage form" is used herein to mean a single or multiple dose form containing a quantity of the micelle containing formulation, said quantity being such that one or more predetermined units are normally required for a single therapeutic administration. In the case of multiple dose forms, such as scored tablets, said predetermined unit will be one fraction, such as a half or quarter of a scored tablet, of the multiple dose form.

It is contemplated that a combination of rapid-acting, short-acting, fast-releasing, long-acting, colorectal release, sustained release, controlled release, pulsatile release, gastric release, enteric release, extended release, timed release or slow release dosage forms may be used in the present invention.

Pharmaceutical Formulation and Administration

For injection, the pharmaceutical composition can be formulated, for reconstitution with an appropriate solution, as, for example and without limitation: freeze dried, rotary dried or spray dried powders; amorphous powders; or granules, precipitates or particulates. For injection, the micelles may also be formulated as suspensions or liquids in the appropriate solutions, such as, by way of example and without limitation, water, aqueous solvents, nonprotic solvents, protic solvents, hydrophilic solvents, hydrophobic solvents, polar solvents, nonpolar solvent and/or combinations thereof, optionally containing stabilizers, pH modifiers, surfactants, bioavailability modifiers and/or combinations thereof. The pharmaceutical composition can be administered in the form of a depot injection or implant preparation which may be formulated in such a manner as to permit a sustained release of the active ingredient. The composition can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or implants. Implants can employ inert materials such as biodegradable polymers or synthetic silicones, for examples, Silastic, silicone rubber-manufactured by the Dow-Corning Corporation.

For inhalation either nasally or orally, the pharmaceutical composition can be formulated as sprays or aerosols containing the appropriate solvents (such as water, aqueous, nonaqueous, polar, nonpolar, hydropic, hydrophilic and/or combinations thereof) and optionally other compounds (stabilizers, perfumes, antimicrobial agents, antioxidants, pH modifiers, surfactants and/or bioavailability modifiers). A propellant such as compressed air, nitrogen, carbon dioxide or hydrocarbon based low boiling solvents (such as butane, propane or others) would be used in an aerosol formulation.

For nasal administration, the same type of formulations used for the inhalation administration may be used. In addition, pastes, ointments or creams may also be used. It is contemplated that bioavailability enhancers such as alcohols or other compounds that enhance the penetration of the micelles into the nasal mucosa may be needed to prepare suitable formulations for nasal administration.

For oral, buccal, and sublingual administration, the pharmaceutical composition of the invention may be administered as either solutions or suspensions in the form of gelcaps, caplets, tablets, capsules or powders. For rectal administration, the compounds of the invention may be administered in the form of suppositories, ointments, enemas, tablets and creams for release of compound in the intestines, sigmoid flexure and/or rectum. It is contemplated that the pharmaceutical formulation can be formulated as, for example and without limitation, freeze dried, rotary dried or spray dried powders; amorphous or crystalline powders; or granules, precipitates or particulates. The solids used can be either free-flowing or compressed. The pharmaceutical formulation can comprise, by way of example and without limitation, water, aqueous solvents, nonprotic solvents, protic solvents, hydrophilic solvents, hydrophobic solvents, polar solvents, nonpolar solvent, emollients and/or combinations thereof, optionally containing stabilizers, pH modifiers, surfactants, perfumes, astringents, cosmetic foundations, pigments, dyes, bioavailability modifiers and/or combinations thereof.

The pharmaceutical composition can also be administered as liquid suspensions or solutions using a sterile liquid, such as an oil, water, an alcohol, or mixtures thereof, with or without the addition of a pharmaceutically suitable surfactants, suspending agent, or emulsifying agent for oral or parenteral administration.

For liquid preparations, the pharmaceutical composition can be formulated suitably with oils, for example, fixed oils, such as peanut oil, sesame oil, cottonseed oil, corn oil and olive oil; fatty acids, such as oleic acid, stearic acid and isostearic acid; and fatty acid esters,

such as ethyl oleate, isopropyl myristate, fatty acid glycerides and acetylated fatty acid glycerides; with alcohols, such as ethanol, isopropanol, hexadecyl alcohol, glycerol and propylene glycol; with glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol; with ethers, such as poly(ethylene glycol) 450, with petroleum hydrocarbons, such as mineral oil and petrolatum; with water, or with mixtures thereof; with or without the addition of a pharmaceutically suitable surfactant, suspending agent or emulsifying agent.

The solid unit dosage form of the invention will comprise DFMO and can be combined with conventional carriers, for example, binders, such as acacia, corn starch or gelatin; disintegrating agents, such as, corn starch, guar gum, potato starch or alginic acid; lubricants, such as, stearic acid or magnesium stearate; and inert fillers, such as lactose, sucrose or corn starch. The solid dosage form may comprise granules. As used herein, the term "granule" is taken to mean particle, crystal, powder, particulate, minitablet, compact or other similar solid forms. The granules used in the invention may display diffusion and/or dissolution controlled release rate profiles according to the components from and processes by which they are made.

For gelcap preparations, the pharmaceutical formulation may include oils, for example, fixed oils, such as peanut oil, sesame oil, cottonseed oil, corn oil and olive oil; fatty acids, such as oleic acid, stearic acid and isostearic acid; and fatty acid esters, such as ethyl oleate, isopropyl myristate, fatty acid glycerides and acetylated fatty acid glycerides; with alcohols, such as ethanol, isopropanol, hexadecyl alcohol, glycerol and propylene glycol; with glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol; with ethers, such as poly(ethylene glycol) 450, with petroleum hydrocarbons, such as mineral oil and petrolatum; with water, or with mixtures thereof; with or without the addition of a pharmaceutically suitable surfactant, suspending agent or emulsifying agent.

Oils can also be employed in the preparation of formulations of the soft gelatin type. Water, saline, aqueous dextrose and related sugar solutions, and glycerols may be employed in the preparation of suspension formulations which may suitably contain suspending agents, such as pectin, carbomers, methyl cellulose, hydroxypropyl cellulose or carboxymethyl cellulose, as well as buffers and preservatives. Soaps and synthetic detergents may be employed as surfactants and as vehicles for detergent compositions. Suitable soaps include fatty acid alkali metal, ammonium, and triethanolamine salts. Suitable detergents include cationic detergents, for example, dimethyl dialkyl ammonium halides, alkyl pyridinium halides, and alkylamine acetates; anionic detergents, for example, alkyl, aryl and olefic

sulfonates, alkyl, olefin, ether and monoglyceride sulfates, and sulfosuccinates; nonionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and poly(oxyethylene)-*block*-poly(oxypropylene) copolymers; and amphoteric detergents, for example, alkyl β-aminopropionates and 2-alkylimidazoline quaternary ammonium salts; and mixtures thereof.

The formulas may also comprise absorbents, antioxidants, buffering agents, colorants, flavorants, sweetening agents, tablet antiadherents, tablet binders, tablet and capsule diluents, tablet direct compression excipients, tablet disintegrants, tablet glidants, tablet lubricants, tablet or capsule opaquants and/or tablet polishing agents.

As used herein, the term "adsorbent" is intended to mean an agent capable of holding other molecules onto its surface by physical or chemical (chemisorption) means. Such compounds include, by way of example and without limitation, powdered and activated charcoal and the like.

As used herein, the term "antioxidant" is intended to mean an agent which inhibits oxidation and thus is used to prevent the deterioration of preparations by the oxidative process. Such compounds include, by way of example and without limitation, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophophorous acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium bisulfite, sodium formaldehyde sulfoxylate and sodium metabisulfite and the like.

As used herein, the term "buffering agent" is intended to mean a compound used to resist change in pH upon dilution or addition of acid or alkali. Such compounds include, by way of example and without limitation, potassium metaphosphate, potassium phosphate, monobasic sodium acetate and sodium citrate anhydrous and dihydrate and the like.

As used herein, the term "colorant" is intended to mean a compound used to impart color to liquid and solid (e.g., tablets and capsules) pharmaceutical preparations. Such compounds include, by way of example and without limitation, FD&C Red No. 3, FD&C Red No. 20, FD&C Yellow No. 6, FD&C Blue No. 2, D&C Green No. 5, D&C Orange No. 5, D&C Red No. 8, caramel, and ferric oxide, red and the like.

As used herein, the term "flavorant" is intended to mean a compound used to impart a pleasant flavor and often odor to a pharmaceutical preparation. In addition to the natural flavorants, many synthetic flavorants are also used. Such compounds include, by way of example and without limitation, anise oil, cinnamon oil, cocoa, menthol, orange oil, peppermint oil and vanillin and the like.

As used herein, the term "sweetening agent" is intended to mean a compound used to impart sweetness to a preparation. Such compounds include, by way of example and without limitation, aspartame, dextrose, glycerin, mannitol, saccharin sodium, sorbitol and sucrose and the like.

As used herein, the term "tablet antiadherents" is intended to mean agents which prevent the sticking of table formulation ingredients to punches and dies in a tableting machine during production. Such compounds include, by way of example and without limitation, magnesium stearate and talc and the like.

As used herein, the term "tablet binders" is intended to mean substances used to cause adhesion of powder particles in table granulations. Such compounds include, by way of example and without limitation, acacia, alginic acid, carboxymethylcellulose sodium, compressible sugar (e.g., "NUTAB"), ethylcellulose, gelatin, liquid glucose, methylcellulose, povidone and pregelatinized starch and the like.

As used herein, the term "tablet and capsule diluent" is intended to mean inert substances used as fillers to create the desired bulk, flow properties, and compression characteristics in the preparation of tablets and capsules. Such compounds include, by way of example and without limitation, dibasic calcium phosphate, kaolin, lactose, mannitol, microcrystalline cellulose, powdered cellulose, precipitated calcium carbonate, sorbitol, and starch and the like.

As used herein, the term "tablet direct compression excipient" is intended to mean a compound used in direct compression tablet formulations. Such compounds include, by way of example and without limitation, dibasic calcium phosphate (e.g., Ditab) and the like.

As used herein, the term "tablet disintegrant" is intended to mean a compound used in solid dosage forms to promote the disruption of the solid mass into smaller particles which are more readily dispersed or dissolved. Such compounds include, by way of example and without limitation, alginic acid, carboxymethylcellulose calcium, microcrystalline cellulose (e.g., "AVICEL"), polacrilin potassium (e.g., "AMBERLITE"), sodium alginate, sodium starch glycollate, and starch and the like.

As used herein, the term "tablet glidant" is intended to mean agents used in tablet and capsule formulations to reduce friction during tablet compression. Such compounds include, by way of example and without limitation, colloidal silica, cornstarch, and talc and the like.

As used herein, the term "tablet lubricant" is intended to mean substances used in tablet formulations to reduce friction during tablet compression. Such compounds include,

by way of example and without limitation, calcium stearate, magnesium stearate, mineral oil, stearic acid, and zinc stearate and the like.

As used herein, the term "tablet/capsule opaquant" is intended to mean a compound used to render a capsule or a tablet coating opaque. May be used alone or in combination with a colorant. Such compounds include, by way of example and without limitation, titanium dioxide and the like.

As used herein, the term "tablet polishing agent" is intended to mean a compound used to impart an attractive sheen to coated tablets. Such compounds include, by way of example and without limitation, carnauba wax, and white wax and the like.

It should be understood, that compounds used in the art of pharmaceutical formulation generally serve a variety of functions or purposes. Thus, if a compound named herein is mentioned only once or is used to define more than one term herein, its purpose or function should not be construed as being limited solely to that (those) named purpose(s) or function(s).

The course and duration of administration of and the dosage requirements for the formulation of the present invention will vary according to the subject being treated, the formulation used, the method of administration used, the severity and type of colorectal cancer being treated, the coadministration of other drugs and other factors. When used for the prevention of cancer, tumors or other related neoplastic disorders, DFMO will generally be administered chronically and at lower doses than those used for their treatment.

Although each unit dosage form contains therapeutically effective amounts of DFMO, it may be necessary to administer more than one such unit dosage form in order to obtain the full therapeutic benefit of the DFMO. More particularly, since DFMO may require moderately high doses, *vide supra*, for preventing and treating cancer, it is very likely that more than one unit dosage will need to be administered to a patient in order to obtain the full therapeutic benefit of DFMO.

For example, consider that the average 70 Kg man has a body surface area of 1.73 m^2. If DFMO is administered at a dosage of up to about 3 g/m^2/day, the minimum toxic dose (MD_{50}) for the prevention of colorectal cancer, then a patient would have to receive about 5 g of DFMO/day, about 10 tablets containing 0.5 g of DFMO. Correspondingly, if the dosage administered is about 0.25 g/m^2/day, the minimum effective dose (ED_{50}) for the prevention of colorectal cancer, then a patient would have to receive about 0.4 g/day, about 1 tablet containing 0.5 g of DFMO.

General

As used herein, the term DFMO is intended to mean a non-racemic preparation of alpha-difluoromethylornithine in its pharmaceutically acceptable salt and/or isomeric forms. (+)-DFMO is intended to mean alpha-difluoromethylornithine having the (D)-configuration around the alpha-carbon which is the only chiral atom present in the molecule. (-)-DFMO is intended to mean alpha-difluoromethylornithine having the (L)-configuration around the alpha-carbon. (+/-)-DFMO is intended to mean racemic alpha-difluoromethylornithine .

Methods for the preparation of (+)-DFMO and (-)-DFMO are known. U.S. 4,330,559, the disclosure of which is hereby incorporated by reference in its entirety, discloses a method for the preparation of optically pure DFMO wherein racemic DFMO dihydrochloride is reacted with sodium methylate to form 3-amino-3-difluoromethyl-2-piperidone (DFMO-pip) which is subsequently crystallized in the presence of (-)-binaphthyl phosphoric acid ((-)-BNPA) to yield (-)-DFMO-pip:(-)-BNPA 1:1 addition salt crystals leaving the (+)-DFMO-pip:(-)-BNPA addition salt in solution. Following repeated recrystallization and acidification, (-)-DFMO-pip is obtained in optically pure form. The (-)-DFMO-pip is then hydrolyzed to yield (+)-DFMO. The enantiopode (+)-DFMO may be prepared according to the above procedure by employing (+)-BNPA to preferentially form the diastereomeric (+)-DFMO-pip:(+)-BNPA 1:1 addition salt crystals.

Wagner, et al. (1987), the disclosure of which is hereby incorporated by reference in its entirety, discloses a reverse phase liquid chromatographic method for the resolution of racemic DFMO to yield each enantiomer of DFMO in optically pure form.

Aldous et al. (1986) discloses a gas chromatographic analytical method for the resolution of racemic DFMO to yield each enantiomer of DFMO in optically pure form.

The compounds herein described may have asymmetric centers. All chiral, diastereomeric, and racemic forms are included in the present invention. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention unless the specific stereochemistry or isomer form is specifically indicated. It will be appreciated that certain compounds of the present invention contain an asymmetrically substituted carbon atom, and may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis, from optically active starting materials. Also, it is realized that cis and trans geometric

isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Following long-standing patent law convention, the terms "a" and "an" mean "one or more" when used in this specification.

One aspect of the present invention provides a pharmaceutical composition having enhanced pharmacological activity relative to racemic DFMO for treating and controlling the growth of cancer, tumors and related neoplastic disorders, the composition comprising a ratio of (+)-DFMO: (-)-DFMO of about 5 % to about 45 %: about 95 % to about 55 % by weight, respectively.

In another aspect, the present invention provides a pharmaceutical composition having enhanced pharmacological activity relative to racemic DFMO for treating and controlling the growth of cancer, tumors and related neoplastic disorders, the composition comprising a ratio of (+)-DFMO: (-)-DFMO of about 25%: about 75% by weight, respectively.

In yet another aspect, the present invention provides a method of reducing the risk of reoccurrence of cancer, tumors and/or related neoplastic disorders comprising administering to a patient a therapeutic amount of a pharmaceutical composition comprising (-)-DFMO or a defined non-racemic ratio of (+)-DFMO: (-)-DFMO, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier; the cancer, tumors or related neoplastic disorders being selected from the group consisting of: neuroblastoma, colon carcinoma, leukemia, hepatoma, mammary sarcoma, small cell lung cancer, Lewis lung cancer, pancreatic cancer, B16 murine melanoma, M3 murine adenocarcinoma, bladder carcinoma, endocervical carcinoma, epithelial cancer, chemically induced cancer, metastatic colorectal cancer, refractory childhood leukemia, cervical intra epithelial neoplasia, hematological malignancies, acute and chronic myeloid leukemia, recurrent glioma, glioblastoma, solid tumor, lymphoma, mammary carcinoma, Barrett's esophagus, oral leukoplakia, premalignant polyps, tamoxifen resistant breast cancer and estrogen independent breast cancer; and the defined non-racemic ratio of (+)-DFMO: (-)-DFMO being about 5% to about 45%: about 55% to about 95% by weight, respectively.

Unless otherwise indicated, all chemicals were purchased from Ilex Oncology, Inc. (San Antonio, TX), or Aldrich Chemicals (Milwaukee, WI).

EXAMPLE 1
PREPARATION OF (+)- and (-)-DFMO
BY CRYSTALLIZATION

3-Amino-3-difluoromethyl-2-piperidone: To a solution of methyl-2-difluoromethyl-2,5-diaminopentanoate-dihydrochloride (2.7 g) in dry methanol (30 ml) is added under nitrogen 2 equivalents of sodium methylate in methanol (0.46 g of sodium in 20 ml of methanol). The reaction mixture is stirred for 3 hours at room temperature then the solvent is evaporated under reduced pressure. The residue is extracted with ether to yield crude 3-amino-3-difluoromethyl-2-piperidone which is purified either by crystallization from $CHCl_3$/pentane: (m.p.: 149°C.) or by distillation (b.p. 135°C./0.05 mmHg).

(—)- and (+)- 3-Amino-3 difluoromethyl-2-piperidone hydrochloride: To a solution of (—)-binaphthylphosphoric acid ((-)-BNPA) (1.27 g) in hot ethanol (50 ml) is added a solution of (±)-3-amino-3-difluoromethyl-2-piperidone (0.546 mg) in hot ethanol (5 ml). On cooling, crystals separate. The reaction mixture is then let stand at 4°C. overnight. The precipitate is filtered off, washed with ethanol and diethyl ether to give 0.54 g of (—)-binaphthylphosphoric salt ([α]ρ=—409°C. =0.3, MeOH mp: 300°C.). Recrystallization of the mother liquor yields 0.15 g of (—)- binaphthylphosphoric salt. Concentration of the filtrate gives 1.1 g of a sticky material which is treated with HC1 3 M at room temperature for 3 hours. The (—)-BNPA is filtered off and the filtrate concentrated under reduced pressure. Recrystallization of the residue (320 mg) in ethanol affords (+)-3-amino-3-difluoromethyl-2-piperidonemonohydrochloride (160 mg) (([α]ρ=+18°6, C=1, MeOH) m.p.: 238°C.). Treated in the same condition the (—)-BNPA salt (436 mg) gives (—)-3-amino-3-difluoromethyl-2-piperidone monohydrochloride (137mg) which is crystallized in ethanol (67 mg) (([α]ρ=—19°, C=1.02, MeOH; mp=240°C.dec.).

(—)- and (+)-2-difluoromethyl-2,5-diamino pentanoic acid monohydrochloride:

(—)-3-Difluoromethyl-3-amino-2- piperidone hydrochloride (60 mg) is heated in HCl 6 M (4 ml) at reflux for 12 hours. After concentration under reduced pressure, the residue is dissolved in water and the pH of the solution is adjusted to 4.5 with a solution of NEt_3. The solution is then concentrated under reduced pressure and the residue extracted many times with chloroform and then recrystallized from H_2O/EtOH to give (+)-2-difluoromethyl-2,5-diamino pentanoic acid monohydrochloride (54 mg) (([α]ρ=+6°, C=0,48, MeOH;

mp≥240°C.). By an identical treatment, (+)-3-difluoromethyl-3-amino-2-piperidone hydrochloride (96 mg) gives (—)-2-difluoromethyl-2,5-diaminopentanoic acid monohydrochloride (56 mg) (([α]ρ=—10°, C=0.7, MeOH, mp≥244°).

EXAMPLE 2
PREPARATION OF (-)-DFMO BY ENZYMATIC RESOLUTION

The following method is the same as that disclosed by Au et al. (1990), the disclosure of which is hereby incorporated in its entirety.

Racemic 3-amino-3-difluoromethyl-2-piperidone (250 mg) is treated with L-alpha-amino-ϵ-caprolactam hydrolase in Tris-HC1 buffer (0.2M, pH 8.5) containing $MnC1_2$ (1 M). The mixture is stirred for 48 h and then extracted with chloroform. The aqueous layer is separated, acidified with 6N HC1 and concentrated to dryness. The residue is recrystallized from a water-ethanol mixture to yield (-)-DFMO (36 mg).

EXAMPLE 3
DETERMINATION OF (+)- AND (-)-DFMO IN BODY FLUIDS

Instrumentation: A gas chromatograph (5730A, Hewlett Packard, Pittsburgh, PA, USA), equipped with an electron capture detector (ecd with pulsed variable frequency and ^{63}Ni as radioactive source) and a recorder-integrator (Hewlett Packard 3380A) is employed. The sample injection is made (Hewlett Packard 7672A autosampler) in the splitness mode (purge delay 30 sec.) on a Chirasil-L-Val capillary fused silica column (Chrompack: WCOT 25 m x 0.22 mm ID, film thickness 0.12, μm). The elution of the two enantiomers of α-DFMO and the internal standard (IS is MFMO:(+),-)-α-monofluoromethylornithine: MDL 71.919) is realized with argon-methane 5% (quality HP) as carrier gas (column pressure 0.6 atm) and with a temperature gradient ranging from 140° to 190°C at a rate of 2°C/min, following a 2 min. isothermal period. The injection portion has a temperature of 250°C and the detector temperature is kept at 350°C.

Plasma samples: To 100 μl of human blank plasma is added known quantities of (+)-αDFMO (MEL 71.872) or (-)-αDFMO (MLD 71.871) and (+, -)-MFML (IS). Plasma proteins are precipitated with 400 μl of methanol and the mixture is vigorously shaken. The sample is centrifuged for 15 min at 5000 rpm ("SORVALL®" Instrument GLC 4-Dupont).

The supernatant is transferred to another vial and evaporated to dryness with a stream of nitrogen. One hundred μl of HBF and 50 μl of HFBA are added to the residue, and the reaction mixture is allowed to stand for 1 h at room temperature. After evaporation of the volatile reagents under nitrogen, the residue is partitioned between dichloromethane (4 ml) and 0.1 M sodium phosphate buffer pH = 5.8 (1 ml). The organic layer is isolated after centrifugation for 10 min at 3000 rpm and further washed with 1 ml of phosphate buffer pH=5.8. The final organic phase is evaporated to dryness and reconstituted with 400 μl of ethyl acetate and transferred into autosampler vials. One μl of this solution is then injected onto the GC column.

Urine samples: a 100 μl aliquot of 24 h human blank urine is directly evaporated under nitrogen after addition of (+) or (-)-αDFMO and the IS in known quantities. In the same way as with plasma, 100 μl of HFB and 50 μl of HFBA are added and the mixture is allowed to react at room temperature for 1 h. After evaporation of the excess of the reagents, the residue is partitioned between 4 ml of dichloromethane and 1 ml of 0.1 M sodium phosphate buffer pH=5.8. To eliminate interfering acidic constituents of the urine, the organic layer is further washed with 5 mM Tris buffer (pH=8.6). After evaporation, the residue of the organic layer is reconstituted with 400 μl of ethyl acetate. One μl of this solution is injected onto the column.

EXAMPLE 4
DETERMINATION OF (-)-DFMO ACTIVITY IN ANIMAL CANCER AND TUMOR MODELS

Nude mice are implanted s.c. by trocar with fragments of human carcinomas harvested from s.c. growing tumors in nude mice hosts. When tumors are approximately 5 mm x 5 mm in size (usually about ten to twenty days after inoculation), the animals are pair-matched into treatment and control groups. Each group contains 10 tumored mice, each of which is ear-tagged and followed individually throughout the experiment. The administration of drugs or vehicle begins the day the animals are pair-matched (Day 1). The doses, route of drug administration and schedule are selected as appropriate for the study in question. If the MTD dose of an agent is not known, it is determined in an initial dosing experiment in non-tumored mice.

The experiment is usually terminated when control tumors reach a size of 1 g or 2 g for the tumor models. Mice are weighed twice weekly, and tumor measurements are taken

by calipers twice weekly, starting on day 1. These tumor measurements are converted to mg tumor weight by a well-known formula, and from these calculated tumor weights, the termination date can be determined. Upon termination, all mice are weighed, sacrificed, and their tumors excised. Tumors are weighed, and the mean tumor weight per group is calculated. In this model, the mean treated tumor weight/mean control tumor weight x 100% (T/C) subtracted from 100% to give the tumor growth inhibition (TGI) for each group.

The final weight of a given tumor is subtracted from its own weight at the start of treatment on day 1. This difference divided by the initial tumor weight is the % shrinkage. A mean % tumor shrinkage can be calculated from data from the mice in a group that experienced regressions. If the tumor completely disappears in a mouse, this is considered a complete regression or complete tumor shrinkage. If desired, mice with partial or total tumor regressions can be kept alive past the termination date to see whether they live to become long term, tumor-free survivors. Statistics are performed on the data using primarily the log rank-value test.

The above general procedure can be adapted for use in demonstrating the effectiveness of non-racemic DFMO for treating, preventing, controlling the growth of and/or reducing the risk of reoccurrence of the following cancers and tumors: metastatic colorectal cancer, small lung cell cancer, pancreatic cancer, epithelial cancer, neuroflostoma, hepatoma, Lewis Lung carcinoma, cervical cancer, M3 murine adenocarcinoma, endocervical cancer and lymphoma. For each indication, the respective cell line and experimental conditions to be used are known to the skilled artisan.

In the above procedure, a reduction in the growth, number, size, metastasis and/or reoccurrence of cancer or tumor in the animal is deemed a positive response.

EXAMPLE 5
PREPARATION OF (+)- AND (-)-DFMO-CONTAINING TABLET COMPRISING RAPID AND SLOW RELEASE LAYERS

One thousand layered tablets, comprising a slow release layer and a rapid release layer of (-)-α-difluoromethylornithine are prepared as follows:

Slow Release Layer

(a) (-)-α-Difluoromethylornithine: 300.0 gm

(b) Hydroxypropylmethylcellulose (400 cps): 100.0 gm

(c) Mannitol: 100.0 gm

(d) Corn starch: 6.0 gm

(e) Zinc stearate: 3.6 gm

Rapid Release Layer

(f) (+)-α-Difluoromethylornithine: 500.0 gm

(g) Microcrystalline cellulose: 100.0 gm

(h) Starch: 100.0 gm

Using a suitable mixer, the (-)-a-difluoromethylornithine, mannitol and hydroxypropylmethylcellulose are mixed well via geometric dilution. The mixture is mixed in a Fitzmill equipped with a No. 000 screen and granulated using a 5 % starch paste prepared by adding the corn starch to approximately 115 ml of water. Additional water is added as required to make a suitable granulation. The resulting granulation is wet-screened using a No. 2 screen and tray dried at 40°C. to 50°C. for 8 to 12 hours. The dried granulation is ground and passed through a No. 10 screen. Zinc stearate, which has passed through a No. 20 screen, is added to the granulation, mixed well and the resulting slow release granulation reserved for tablet compression.

The (+)-α-difluoromethylornithine for the rapid release layer is milled, if necessary, to obtain a powder having the majority of particles in the range of 10 to 150 microns in size. The milled powder, microcrystalline cellulose and starch are mixed well in a Fitzmill equipped with No. 000 screen and the resulting rapid release mixture reserved for tablet compression.

Using a suitable layer press, such as the Manesty Layer Press, the slow release granulation is added to the adjusted die cavity to provide a layer having a weight of approximately 500 mg. The rapid release granulation is added to the die cavity and the final compression pressure is adjusted to provide a suitable tablet with a total weight of approximately 1.2 g.

EXAMPLE 6
PREPARATION OF (-)-DFMO-CONTAINING TOPICAL SOLUTION

Ethyl alcohol (8 mL) is thoroughly mixed with isopropyl myristate (5 g) and poly(ethylene glycol) 400 (10 g). While mixing add (-)-DFMO (8.5 g) and sufficient purified water to make a total 100 mL volume. This formulation is suitable for topical use as for the treatment or prevention of skin cancer.

EXAMPLE 7
PREPARATION OF (-)-DFMO-CONTAINING LOTION

Isostearic acid (10 g) and stearic acid (8 g) are thoroughly mixed with poloxamer 235 (10 g). While mixing, add to this mixture propylene glycol (10 g) and (-)-DFMO (10 g) until thoroughly mixed. Finally, add with mixing sufficient purified water to make a 100 mL formulation final volume. This lotion formulation is suitable for topical use as for the treatment or prevention of skin melanoma.

EXAMPLE 8
PREPARATION OF (-)-DFMO-CONTAINING TOPICAL SOLUTION

A vehicle (100 g) is prepared by mixing the following ingredients in the specified amounts (weight percent based upon the total vehicle weight): water (68%), ethanol (16%), propylene glycol (5%), dipropylene glycol (5%), benzyl alcohol (4%) and propylene carbonate (2%). Varying amounts of the vehicle (90 to 99.5 g) are then thoroughly mixed with (-)-DFMO (10 to 0.5 g). This formulation is suitable for topical administration as for the treatment or prevention of proliferative skin disorders.

EXAMPLE 9
PREPARATION OF (-)-DFMO-CONTAINING GRANULES FOR DISSOLUTION IN WATER

(-)-DFMO (33 g) is mixed with lactose (6 g) and passed through a fluid energy mill or micronizer to give a particle size of 1-25 microns. Water (35 mL) is added to corn starch (2 g) and blended to prepare a starch paste. The micronized (-)-DFMO-lactose powder, lactose (42.2 g) and corn starch (16.5 g) are well blended. The starch paste is added and the entire mixture blended. The resulting mixture is passed through as No. 12 mesh screen. The resulting granules are dried at 38°C to a moisture content of about 3 % by weight, ground thorough a U.S. Standard No. 12 screen and lubricated by mixing with 0.3 g or zinc stearate. The above formulation is suitable for dissolution in water for oral administration as for the treatment or prevention of Barrett's Esophagus.

EXAMPLE 10
PREPARATION OF (-)-DFMO-CONTAINING RECONSTITUTABLE DRY BEVERAGE BASE

The following ingredients are thoroughly mixed in the amounts specified: Fries & Fries grapefruit flavoring #91470 (5.0 g), fructose USP (30.0 g), aspartame (0.5 g), citric acid (anhydrous, 2.0 g) and (-)-DFMO (30.0 g). The above mixture will generally be administered to a patient by reconstituting 10 % by weight of the final formulation in water (200 mL) to arrive at a unit dose for oral administration as for the treatment or prevention of breast cancer.

EXAMPLE 11
PREPARATION OF (-)-DFMO-CONTAINING ORAL SOLUTION

To distilled water (7.0 mL) is added sodium benzoate (15 mg), and saccharin sodium (18 mg) and the mixture heated to 50-60°C. (-)-DFMO (3.0 g) is added until dissolution. After cooling the solution to 20 - 30° C, add ethanol 0.75 mL, glycerin (0.75 mL), propylene glycol (1.5 mL) and water to a final solution volume of 15 mL. The above formulation is suitable for oral administration as for the treatment or prevention of pancreatic cancer.

EXAMPLE 12
PREPARATION OF (-)-DFMO-CONTAINING CONVENTIONAL RELEASE HARD GELATIN CAPSULES

One thousand two-piece hard gelatin capsules for oral use, each containing 200 mg. of (-)-DFMO are prepared from the following types and amounts of materials:

(-)-DFMO: 200 gm.

Corn starch: 150 gm.

Talc: 75 gm.

Magnesium stearate: 2.5 gm.

The materials are thoroughly mixed and then encapsulated in the usual manner.

Using the procedure above, capsules are similarly prepared containing in 5, 100, and 500 mg. amounts by substituting 5, 100, and 500 gm. of (-)-DFMO for the 200 gm. used above.

EXAMPLE 13
PREPARATION OF (-)-DFMO-CONTAINING TOPICAL OINTMENT

(-)-DFMO: 50 gm.

Methyl glyoxal bis-(guanyl hydrazone): 50 gm.

Liquid petrolatum (heavy): 250 gm.

Wool fat: 200 gm.

White petrolatum q.s.: 1000 gm.

The white petrolatum and wool fat are melted and 100 gm. of liquid petrolatum added thereto. The methyl glyoxal bis-(guanyl hydrazone) and (-)-DFMO are added to the remaining liquid petroleum and the mixture milled until the powder is finely divided and uniformly dispersed. The powder mixture is stirred into the white petrolatum mixture and stirring continued until the ointment congeals.

EXAMPLE 14
PREPARATION OF (-)-DFMO-CONTAINING CREAM

A) (-)-DFMO: 1000 gm.

trans-1,4 diamino-2-butene: 500 gm.

Cetyl alcohol: 600 gm.

Stearyl alcohol: 600 gm.

Aerosol OT: 150 g.

White petrolatum: 3000 gm.

Propylene Glycol: 1000 ml.

Distilled Water q.sl.: 10000 gm.

The (-)-DFMO and trans-1,4-diamino-2-butene are mixed with the white petrolatum and stirred into a melt of the alcohols and propylene glycol. The aerosol OT is dissolved in 5000 cc. of water and an emulsion formed with the petrolatum mix, sufficient water being added to make 10,000 gm.

Optionally, substituting 2,000 grams of dimethylacetamide for 2000 grams of water, or 200-500 grams of dimethylsulfoxide for 200-500 grams of water, a composition is obtained providing better penetration of the active ingredients into the skin.

B) 1000 grams of a topical cream is usefully prepared from the following types and amounts of ingredients:

(-)-DFMO: 100 grams

Hydrocortisone: 10 grams

Tegacid Regular: 150 grams

Spermaceti: 100 grams

Propylene glycol: 50 gm.

Polysorbate 80: 5 gm.

Methylparaben: 1 g.

Deionized water q.s.: 1000 gm.

The (-)-DFMO and hydrocortisone are added to the other components in the same manner described above. This composition, applied topically with occlusive bandage to humans, is particularly effective in treating, preventing and controlling the growth rate of skin cancers. Similar results are obtained by substituting for the 10 grams of hydrocortisone with 0.10 grams of triamcinolone.

EXAMPLE 15
PREPARATION OF (-)-DFMO-CONTAINING CONVENTIONAL RELEASE TABLETS

A) One thousand tablets suitable for oral use prepared in accordance with the following formulation:

(a) (-)-α-Difluoromethylornithine: 500.0 gm

(b) Dicalcium phosphate: 250.0 gm

(c) Methylcellulose, U.S.P. (15 cps): 6.5 gm

(d) Talc: 20.0 gm

(e) Calcium stearate: 2.5 gm

The (-)-α-difluoromethylornithine and dicalcium phosphate are mixed well as a dry powder. The resulting powder is granulated using a 7.5 % aqueous solution of methylcellulose, passed through a No. 8 screen and carefully dried. The dried granules prepared in this fashion are passed through a No. 12 screen, lubricated with the remaining talc and calcium stearate, and compressed into tablets.

B) Another illustrative composition for tablets is as follows:

(a) (-)-DFMO: 200 mg

(b) starch: 43 mg

(c) lactose: 45 mg

(d) magnesium stearate: 2 mg

The granulation obtained upon mixing the lactose with the compound (a) and part of the starch and granulated with starch paste is dried, screened, and mixed with magnesium stearate. The mixture is compressed into tablets weighing 290 mg each.

EXAMPLE 16
PREPARATION OF (-)-DFMO-CONTAINING ORAL SYRUP

One thousand cc. of an aqueous suspension for oral use, containing in each 5 cc. dose 200 mg. of (-)-DFMO is prepared from the following types and amounts of ingredients:

(-)-DFMO: 40 gm.

Citric acid: 2 gm.

Benzoic acid: 1 gm.

Sucrose: 700 gm.

Tragacanth: 5 gm.

Lemon oil: 2 cc.

Deionized water q.s.:

The citric acid, benzoic acid, sucrose, tragacanth, and lemon oil are dispersed in sufficient water to make 850 cc. of solution. The (-)-DFMO is stirred into the syrup until uniformly distributed. Sufficient water is added to make 1000 cc. The composition so prepared is useful in the systemic treatment of various cancers, tumors and related neoplastic disorders in adult humans.

EXAMPLE 17
PREPARATION OF (-)-DFMO-CONTAINING INJECTABLE SUSPENSION

An illustrative composition for an injectable suspension is the following 1 ml ampule for an intramuscular injection.

(a) (-)-2,5-diamino-2-difluoromethyl pentanoic acid: 20 wt. %

(b) polyvinylpyrrolidone: 0.5 wt. %

(c) lecithin: 0.25 wt. %

(d) water for injection to make: 100.0 wt. %

The materials (a)-(d) are mixed, homogenized, and filled into 1 ml ampules which are sealed and autoclaved 20 minutes at 121°C. Each ampule contains 200 mg per ml of compound (a).

EXAMPLE 18
PREPARATION OF (-)-DFMO-CONTAINING INJECTABLE SOLUTION

A sterile aqueous solution for intramuscular use, containing in 1 cc. 75 mg. of (-)-DFMO is prepared from the following types and amounts of materials:

(-)-DFMO: 75 gm.

Methylparaben: 2.5 gm.

Propylparaben: 0.17 gm.

Water for injection q.s.: 1000 cc.

The ingredients are dissolved in the water and the solution sterilized by filtration. The sterile solution is filled into vials and the vials sealed. The composition is useful in the systemic treatment of cancers, tumors and related neoplastic disorders.

EXAMPLE 19
MCF-7 HUMAN BREAST TUMOR ASSAY

Female ovariectomized nude mice are implanted s.c. by trocar with 21 day release 0.25 mg estrogen pellets. The following day the mice are implanted s.c. by trocar fragments of MCF-7 mammary carcinomas harvested from s.c. growing MCF-7 tumors in nude mice hosts. When tumors are approximately 5 mm x 5 mm in size (about twenty days after inoculation), the animals are pair-matched into treatment and control groups, and the estrogen pellets removed. Each group contains 10 tumored mice, each of which is ear-tagged and followed individually throughout the experiment. The administration of drugs or vehicle begins the day the animals are pair-matched (Day 1). The doses, route of drug administration and schedule are selected as appropriate for the study in question. If the MTD dose of an agent is not known, it is determined in an initial dosing experiment in non-tumored mice.

Mice are weighed twice weekly, and tumor measurements are taken by calipers twice weekly, starting on Day 1. These tumor measurements are converted to mg tumor weight by a well-known formula, $L^2xW/2$. The experiment is terminated when control tumors reach a size of 1 gram. Upon termination, all mice are weighed, sacrificed, and their tumors

excised. Tumors are weighed and the mean tumor weight per group is calculated. In these models, the mean treated tumor weight/mean control tumor weight x 100% (T/C) is subtracted from 100% to give the tumor growth inhibition (TGI) for each group.

The final weight of a given tumor is subtracted from its own weight at the start of treatment on Day 1. This difference divided by the initial tumor weight is the % shrinkage. A mean % tumor shrinkage can be calculated from data from the mice in a group that experienced tumor regressions. If the MCF-7 tumor completely disappears in a mouse, this is considered a complete regression or complete tumor shrinkage. If desired, mice with partial or total tumor regressions can be kept alive past the termination date to see whether they live to become long term, tumor-free survivors.

EXAMPLE 20
B16 MURINE MELANOMA ASSAY

B6D2F1 mice receive i.p. inocula of B16 murine melanoma brei prepared from B16 tumors growing s.c. in mice (Day 0). On Day 1, tumored mice are treated with drugs or vehicle control; the route of drug administration and schedule are selected as appropriate for the study in question. If dosing information for agents is not available, the maximum tolerated dose (MTD) is determined in initial dose finding experiments in non-tumored mice. In this experiment, drugs will be given at their MTD and ½ MTD doses i.p. on a daily x 5 schedule.

The mean survival times of all groups are calculated, and results are expressed as mean survival of treated mice/mean survival of control mice (T/C) x 100%. A T/C value of 150 means that the mice in the treated group lived 50% longer than those of the control group; this is sometimes referred to as the increase in life span, or ILS value.

Mice that survive for 60 days are considered long term survivors, or cures, in the B16 model. The universally accepted cut-off for activity in this model, which has been used for years by the NCI, is T/C = 125. Conventional use of B16 over the years has set the following levels of activity: T/C < 125, no activity; T/C = 125-150, weak activity; T/C - 150-200, modest activity; T/C = 200-300, high activity; T/C > 300, with long term survivors; excellent, curative activity.

EXAMPLE 21
P388 LEUKEMIA ASSAY

B6D2F1 mice receive i.p. inocula of P388 murine leukemia prepared by removing ascites fluid containing P388 cells from rumored B6D2F1 mice, centrifuging the cells, and then resuspending the cells in saline. Mice receive $1x10^6$ P388 cells i.p. on Day 0. On Day 1, rumored mice are treated with drugs or vehicle control; the route of drug administration and schedule are selected as appropriate for the study in question. If dosing information for agents is not available, the maximum tolerated dose (MTD) is determined in initial dose finding experiments in non-tumored mice. In a typical experiment, drugs are given at their MTD and ½ MTD doses i.p. on a daily x 5 schedule.

The mean survival times of all groups are calculated, and results are expressed as mean survival of treated mice / mean survival of control mice (T/C) x 100%. A T/C value of 150 means that the mice in the treated group lived 50% longer than those of the control group; this is sometimes referred to as the increase in life span, or ILS value.

Mice that survive for 30 days are considered long term survivors, or cures, in the P388 model. The universally accepted cut-off for activity in this model, has been used for years by the NCI, is T/C = 125. Conventional use of P388 over the years has set the following levels of activity: T/C < 125, no activity; T/C = 125-150, weak activity; T/C = 150-200, modest activity; T/C = 200-300, high activity; T/C > 300, with long term survivors; excellent, curative activity. Statistics are performed on the data using primarily the log rank p-value test.

EXAMPLE 22
EFFECTS OF ENANTIOMERS OF DIFLUOROMETHLORNITHINE ON ORNITTHINE DECARBOXYLASE ACTIVITY

Two model systems were used as proposed. One was ornithine decarboxylase (ODC) synthesized in cell free systems from mouse ODC cDNA, as reported (Glass, *et al.*, 1987). The second system was intact colon cancer derived HCT-116 cells. This cell model has been used in other studies of polyamine metabolism (Ignatenko, *et al.*, 1996). These cells were treated for 24 hours with difluoromethylornithine (DFMO). At various times after removal, cultures were harvested and ODC enzyme activity and intracellular polyamine contents were assessed. Other studies have been reported using DL-DFMO treatment of rat hepatoma tissue culture (HTC) cells (Gerner, *et al.*, 1986).

Cell free system

ODC synthesized from mouse cDNA was incubated for 30 minutes in varying concentrations of L-, D- or DFMO. Reactions were stopped and ODC enzyme activity assessed. Figures 1 (linear abscissa) and 2 (log scale) present the results of studies varying the DFMO concentration for different substrate (ornithine) concentrations. The L- form of DFMO is a more potent inhibitor of ODC activity at low inhibitor/substrate ratios than is the D-form of the inhibitor. The racemic mixture is generally intermediate in potency, although this system does not allow more detailed quantitation of this relationship. Figure 3 shows double reciprocal plots of these results. The K_m for ODC is ~40 μM, which is somewhat higher than that reported for the purified enzyme. It appears as though the mechanism of inhibition of L-DFMO is uncompetitive. D-DFMO may be acting as a competitive inhibitor, but further quantitation of this inhibition is not possible in this system. These observations will be confirmed using a purified enzyme model.

HCT-116 cell model.

Cultures were subcultured and treated for 24 hours with DFMO forms. At that time, media was removed and replaced with fresh medium withou DFMO. Cultures were then harvested at various times after removal of drug. Cells were lysed and lysates were analyzed for ODC activity and polyamine contents. As shown in Figure 4, ODC enzyme activities were very low and no DFMO concentrations effects were evident in this model system. Table 1 shows polyamine contents. Figure 5 displays these contents for cultures harvested immediately after removal of DFMO. The results obtained are similar to those found in the cell free system. Namely, L-DFMO was more potent in reducing cellular polyamine contents than D-DFMO. The racemic mixture was intermediate in potency. All forms suppressed putrescine levels below limits of detection, so that only spermidine contents were plotted in Figure 5. Spermine contents did show some changes, but were not remarkable. Figure 6 displays polyamine contents as a function of time after DFMO removal. Generally, polyamine contents recovered more quickly in the D-DFMO treated cultures than in the L-DFMO treated cells.

Table 1

D-DFMO

Control				
Day	0	1	2	3
Put	2.5469	0	0	0
Spd	6.0712	0	0.9494	1.0670
Spm	4.7662	0	1.1616	1.0294
D-0.1 mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	8.0441	0.1778	1.2964	3.7885
Spm	8.7214	0	1.4819	3.9362
D-0.5 mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	3.4182	0.5125	3.1972	0.7063
Spm	7.1306	0	5.1875	0.7368
D-1.0 mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	2.7496	0	0.8545	2.2008
Spm	6.9506	0	2.0762	2.6842
D-5.0 mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	0.9871	0.4528	0.4173	2.4598
Spm	6.9235	0	3.5550	0

Table 2

L-DFMO

Control	
put	61887
spd	2722145
spm	1807291
dah	328823

L-0.1mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	1.3831	0	1.1881	1.5579
Spm	7.6556	0.4704	1.9260	1.6659
L-0.5mM				
Day	0	1	2	3
Put	0	0	0	
Spd	0	0.2223	0	
Spm	0.1074	2.1201	0.5166	
L-1.0mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	0.9602	0	0.8955	0
Spm	6.3206	0	2.6800	0
L-5.0mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	1.0219	0	0.1061	0
Spm	7.4654	0	0.3659	0

Table 3

Racemic DFMO

R-0.1mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	2.50441	0	1.2101	0.8234
Spm	7.1005	0	1.4153	1.4049
R-0.5mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	0.5808	0	2.6979	0.8690
Spm	6.1617	0	10.4913	1.2184
R-1.0mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	0.5737	0	0	1.6017
Spm	7.6798	0	0.2446	3.5312
R-5.0mM				
Day	0	1	2	3
Put	0	0	0	0
Spd	1.3690	0	0	0
Spm	6.0516	0	1.6497	1.0925

These studies demonstrate that L-DFMO is a more potent inhibitor of ODC and polyamine synthesis than is D-DFMO, both in cell free and intact cell systems. A rouge estimate, based on an evaluation of concentrations to inhibit polyamine synthesis by 50% in these models, supports the observation that the L-form is at least 10 times more potent than the D-form of DFMO. The racemic form of DFMO is generally intermediate in potency between the L- and D-forms.

The recovery of polyamine synthetic activity after removal of DFMO appears different in cells treated with the L- and D-forms. The mechanisms of inhibition by the L- and D-forms of DFMO may also be different, and said differences may be used to increase the relative availability of one form over the other. through use in conjection with tther pharmacologically active agents.

The above is a detailed description of a particular embodiment of the invention. It is recognized that departures from the disclosed embodiment may be made within the scope of the invention and that obvious modifications will occur to a person skilled in the art. The full scope of the invention is set out in the claims that follow and their equivalents. Accordingly, the claims and specification should not be construed to unduly narrow the full scope of protection to which the invention is entitled.

Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments where are disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the invention. All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. It will be apparent that certain compounds which are both physiologically and chemically related may be substituted for the therapeutic compound described herein while the same or similar results are achieved.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising (-)-DFMO or a defined non-racemic ratio of (+)-DFMO: (-)-DFMO, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier, the pharmaceutical composition having enhanced pharmacological activity relative to a pharmaceutical composition comprising racemic DFMO.

2. A pharmaceutical composition as described in Claim 1 where the defined non-racemic ratio of (+)-DFMO: (-)-DFMO is about 5% to about 45%: about 95% to about 55% by weight, respectively.

3. A pharmaceutical composition as described in Claim 1 where the defined non-racemic ratio of (+)-DFMO: (-)-DFMO is about 0% to about 5%: about 99% to about 95 %, respectively.

4. A medicament for use in the treatment of cancer, tumors and/or related neoplastic disorders comprising a therapeutic amount of a pharmaceutical composition comprising (-)-DFMO or a defined non-racemic ratio of (+)-DFMO: (-)-DFMO, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier.

5. A medicament for use in the prevention of cancer, tumors and/or related neoplastic disorders comprising administering to a patient a therapeutic amount of a pharmaceutical composition comprising (-)-DFMO or a defined non-racemic ratio of (+)-DFMO: (-)-DFMO, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier.

6. A medicament for use in controlling the growth of cancer, tumors and/or related neoplastic disorders comprising administering to a patient a therapeutic amount of a pharmaceutical composition comprising (-)-DFMO or a defined non-racemic ratio of (+)-DFMO: (-)-DFMO, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier.

7. A medicament for use in reducing the risk of reoccurrence of cancer, tumors and/or related neoplastic disorders comprising administering to a patient a therapeutic amount of a pharmaceutical composition comprising (-)-DFMO or a defIned non-racemic ratio of (+)-DFMO: (-)-DFMO, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier.

8. The medicament of claim 4, 5, 6 or 7, wherein the cancer, tumors or related neoplastic disorders is selected from the group consisting of:
neuroblastoma, colon carcinoma, leukemia, hepatoma, mammary sarcoma, small cell lung cancer, Lewis lung cancer, pancreatic cancer, B16 murine melanoma, M3 murine adenocarcinoma, bladder carcinoma, endocervical carcinoma, epithelial cancer, chemically induced cancer, metastatic colorectal cancer, refractory childhood leukemia, cervical intraepithelial neoplasia, hematological malignancies, acute and chronic myeloid leukemia, recurrent glioma, glioblastoma, solid tumor, lymphoma, mammary carcinoma, Barrett's esophagus, oral leukoplakia, premalignnnt polyps, tamoxifen resistant breast cancer and estrogen independent breast cancer.

9. The medicament of claim 4, 5, 6 or 7, where the defined non-racemic ratio of (+)-DFMO: (-)-DFMO is about 5:95 to about 45:55 by weight, respectively.

10. The method as described in claim 6, 7, 8 or 9, where the defined non-racemic ratio of (+)-DFMO: (-)-DFMO is about 1:99 to about 5:95 by weight respectively.

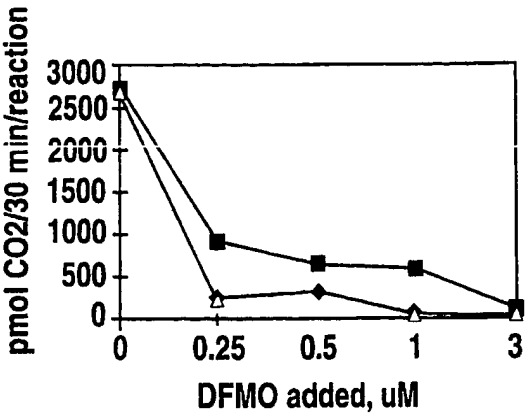


Fig. 1A

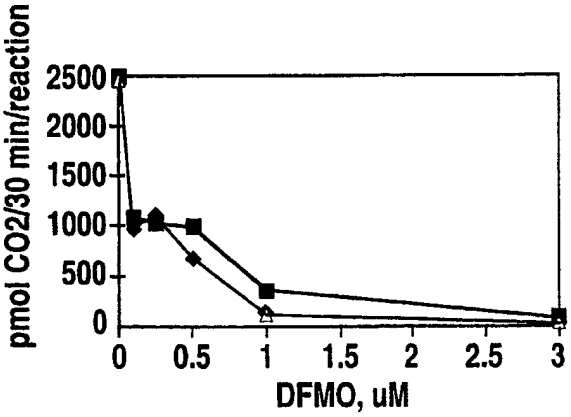


Fig. 1B

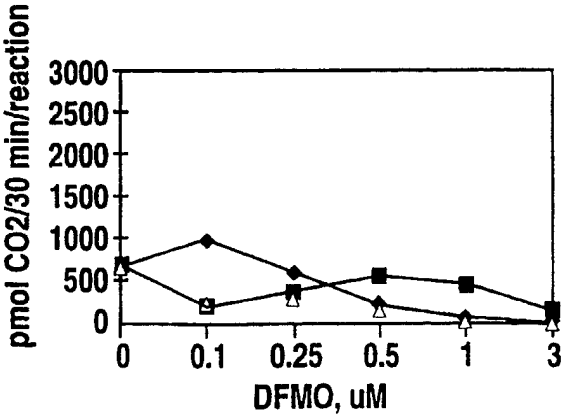


Fig. 1C

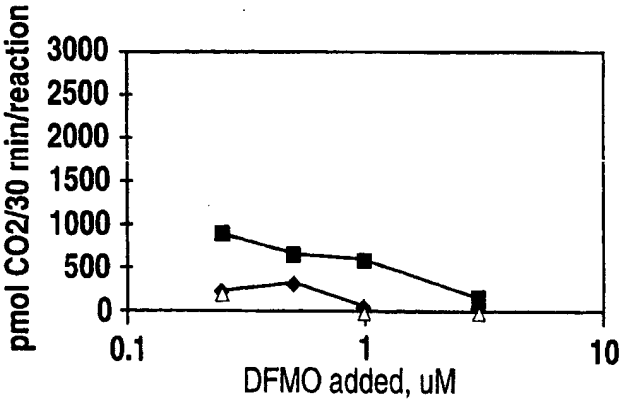


Fig. 2A

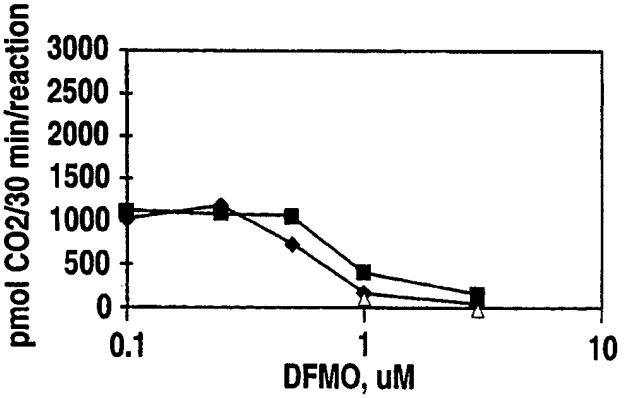


Fig. 2B

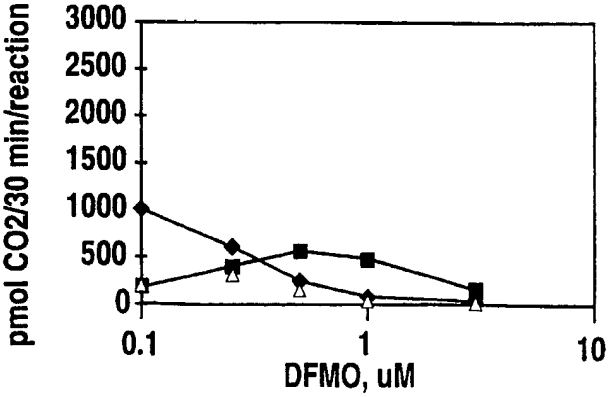


Fig. 2C

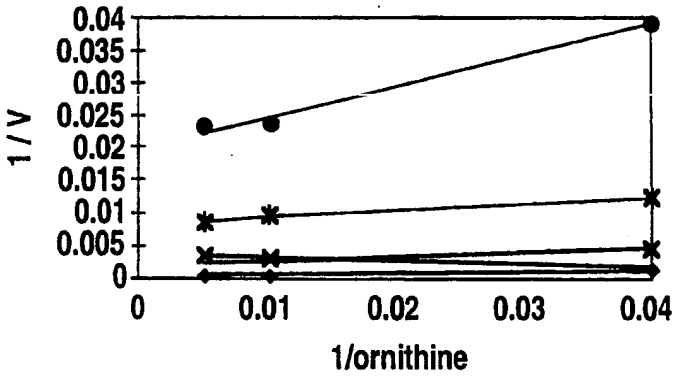


Fig. 3A

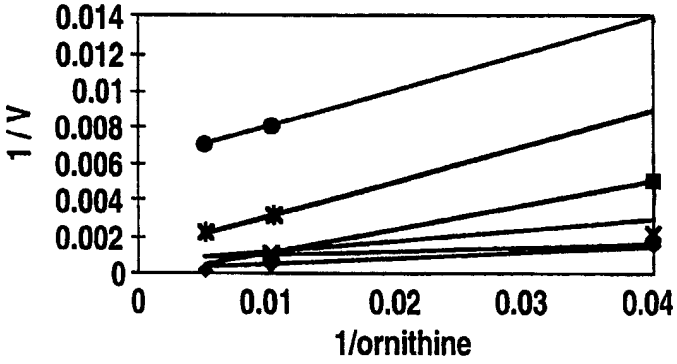


Fig.3B

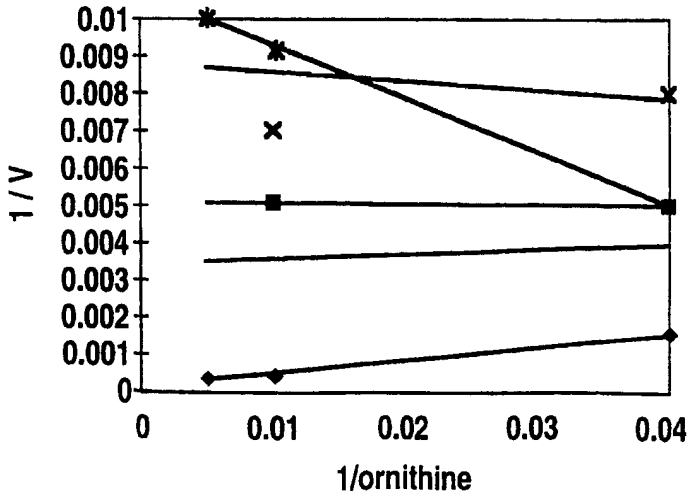
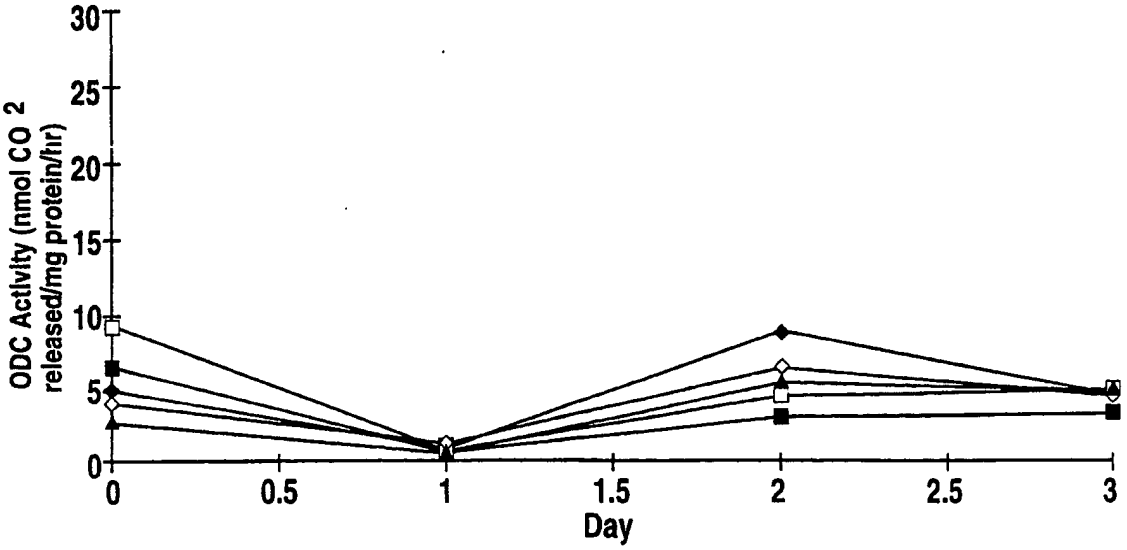


Fig. 3C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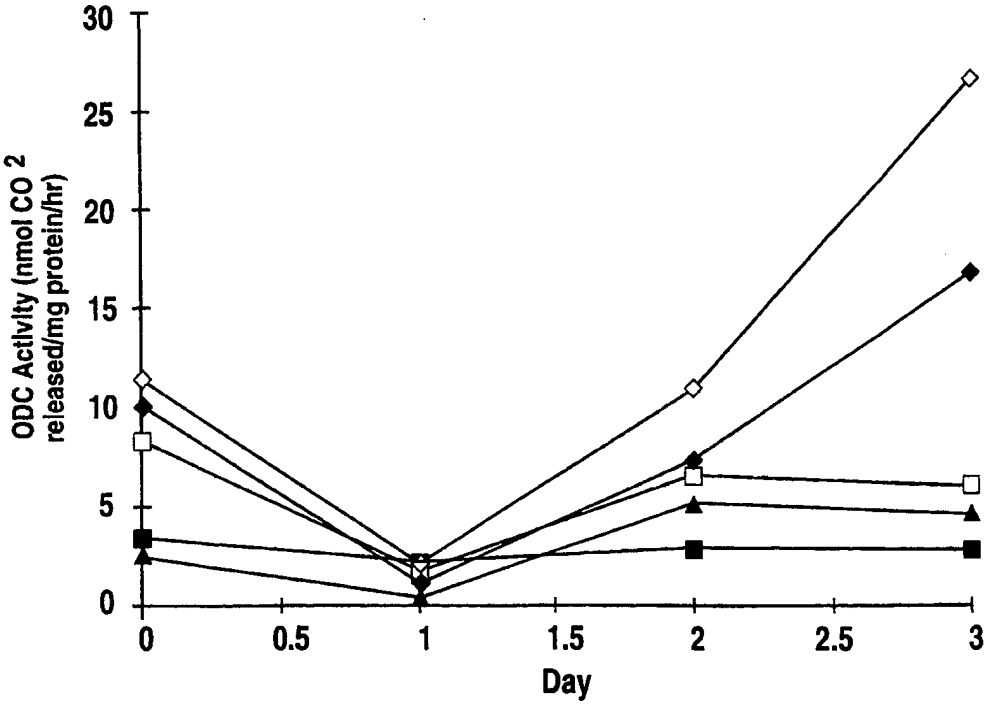
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
Day

Fig. 4a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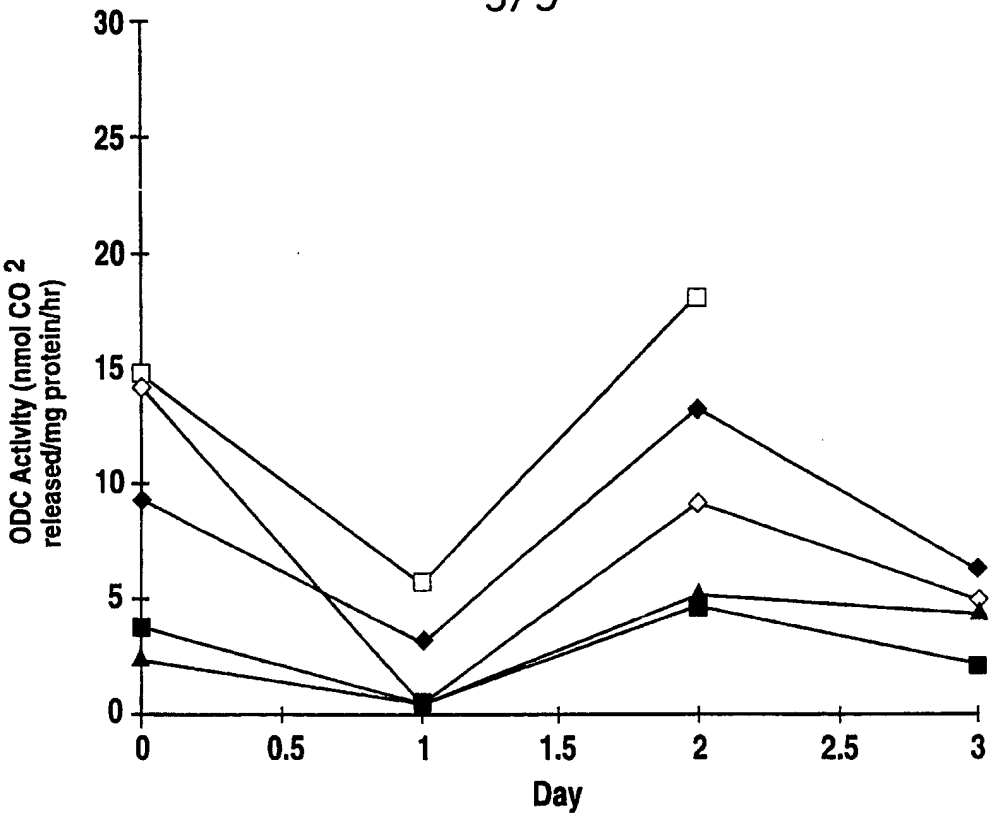
1
1.5
2
2.5
3
Day

Fig. 4b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ODC Activity (nmol CO 2 released/mg protein/hr)
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1
1.5
2
2.5
3
Day

Fig. 4c

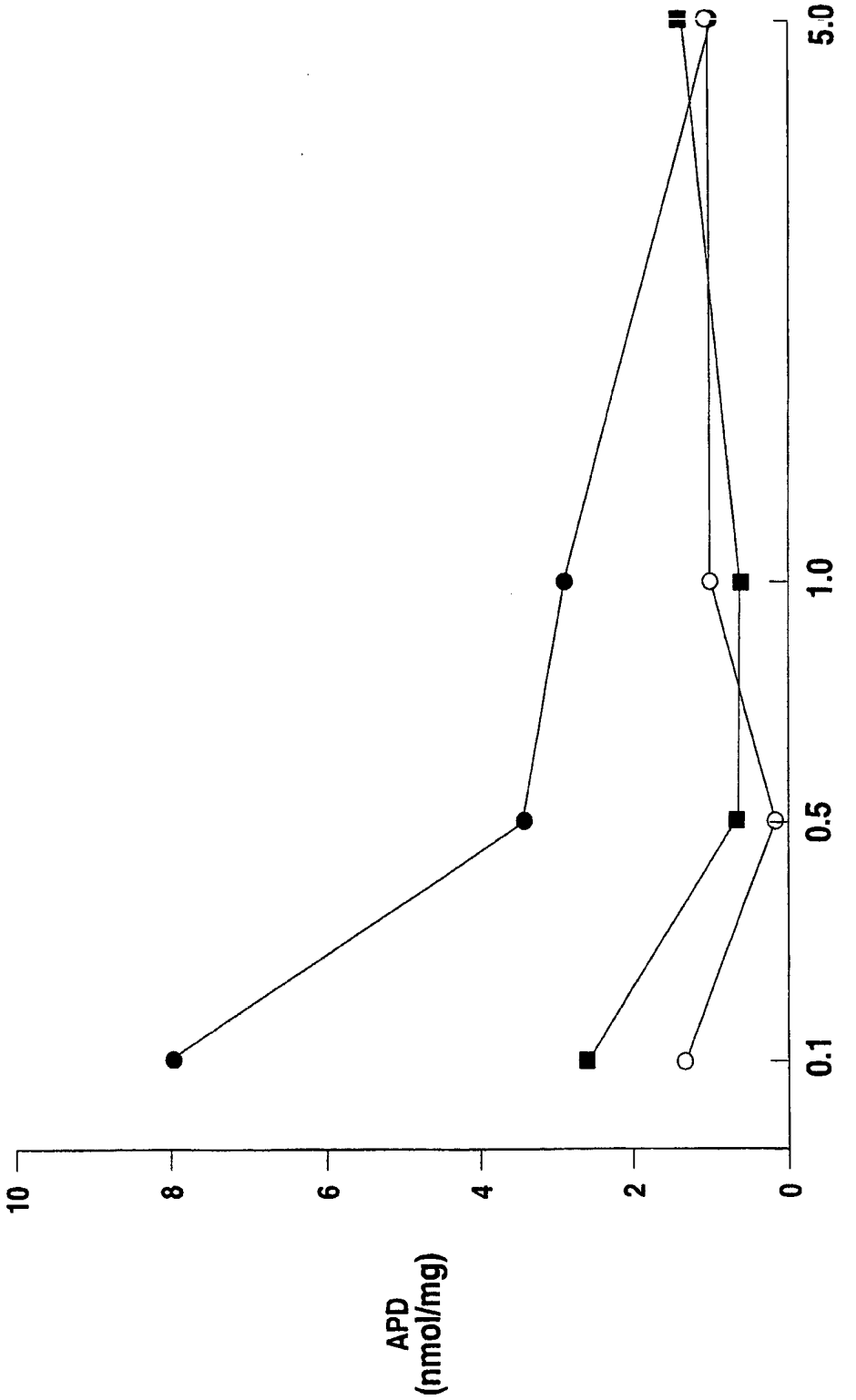

Fig. 5

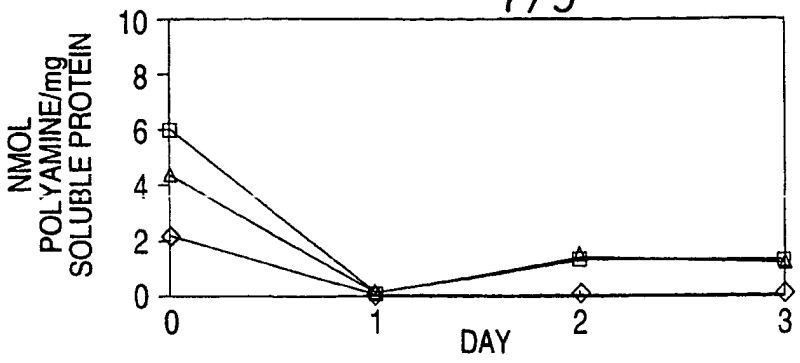

Fig. 6a

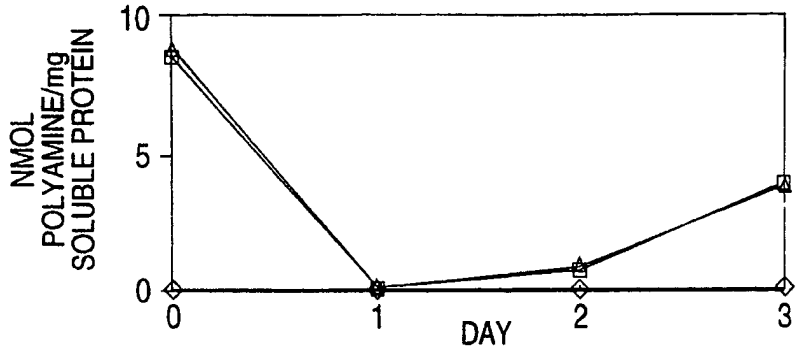

Fig. 6b

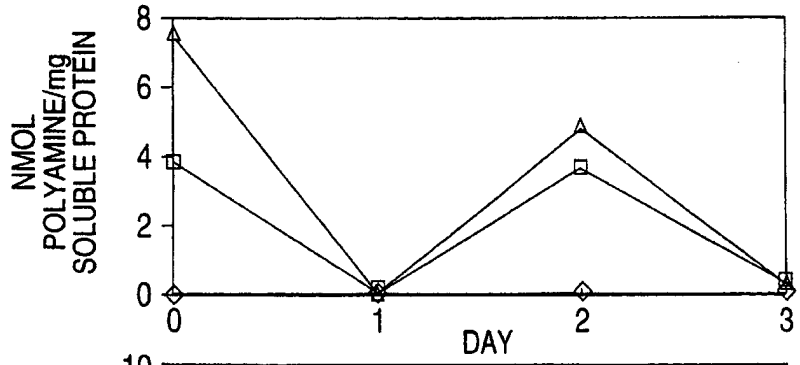

Fig. 6c

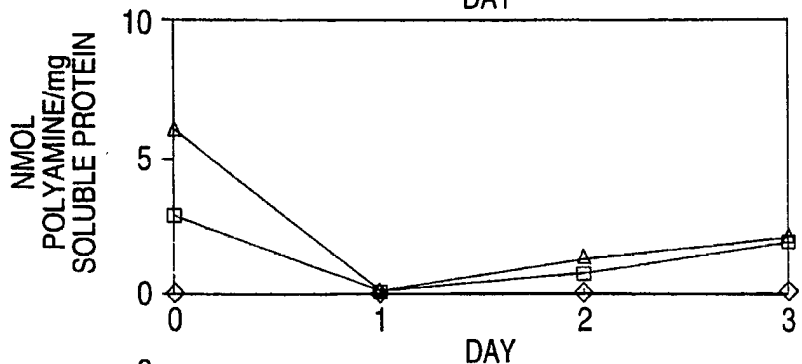

Fig. 6d

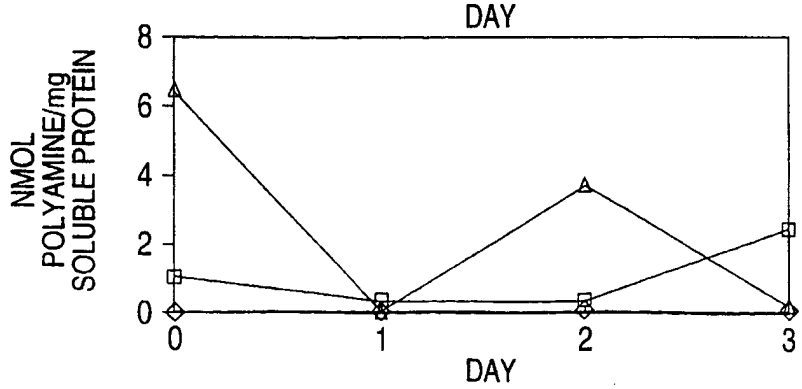

Fig. 6e

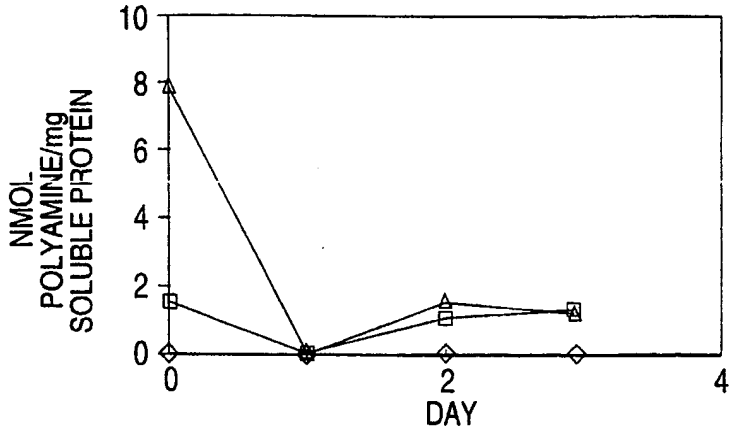

Fig. 7a

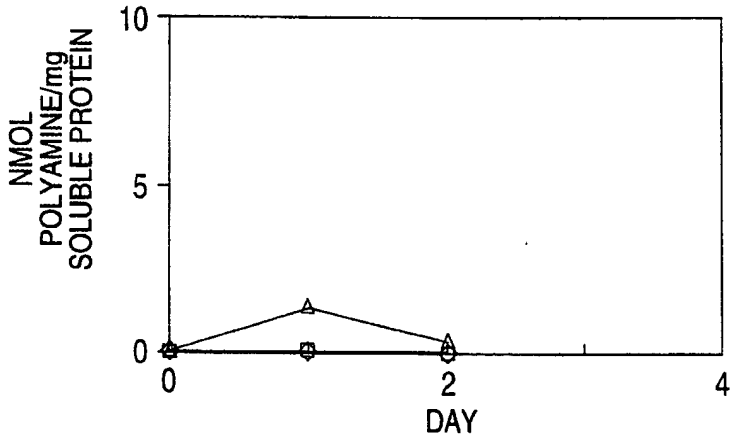

Fig. 7b

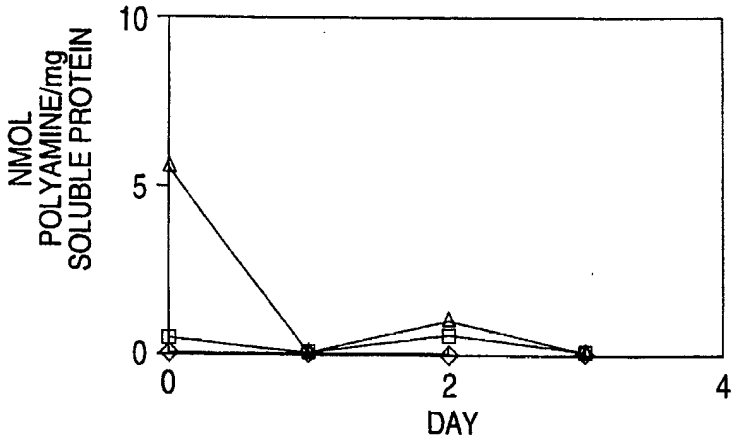

Fig. 7c

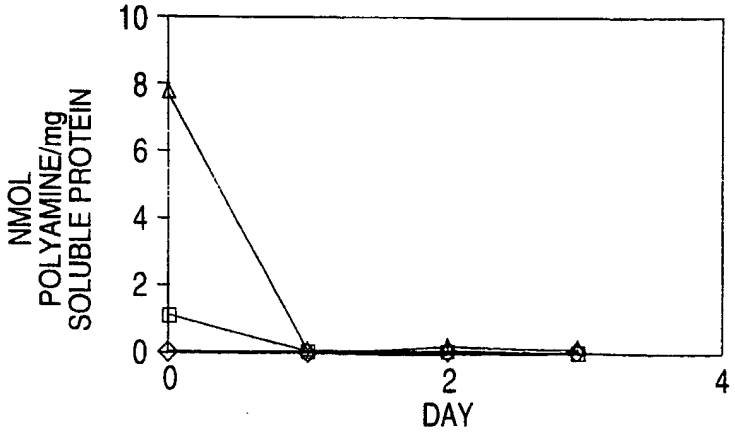

Fig. 7d

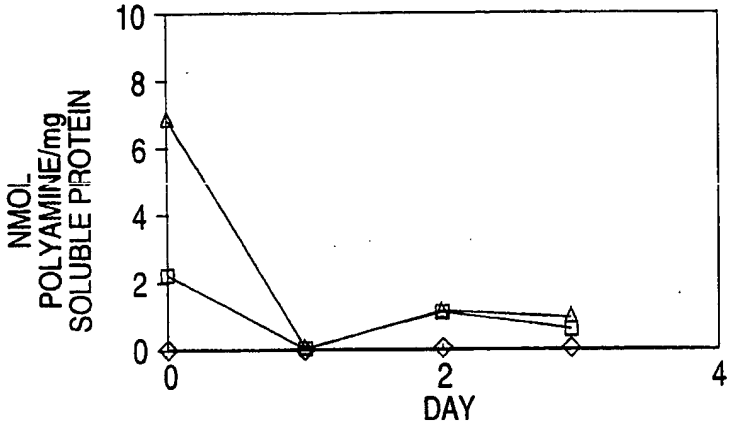

Fig. 8a

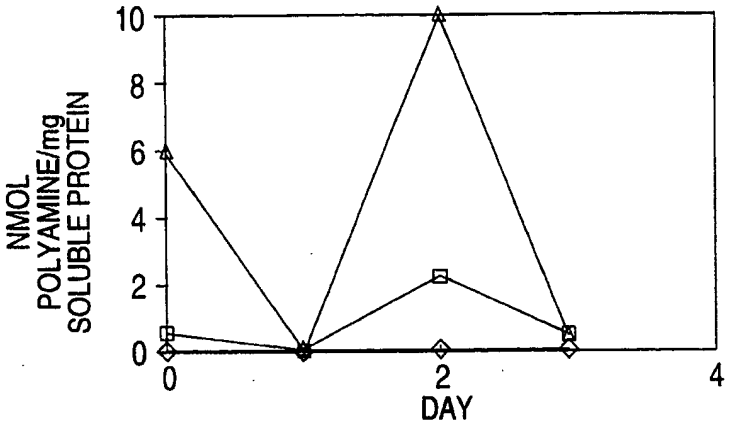

Fig. 8b

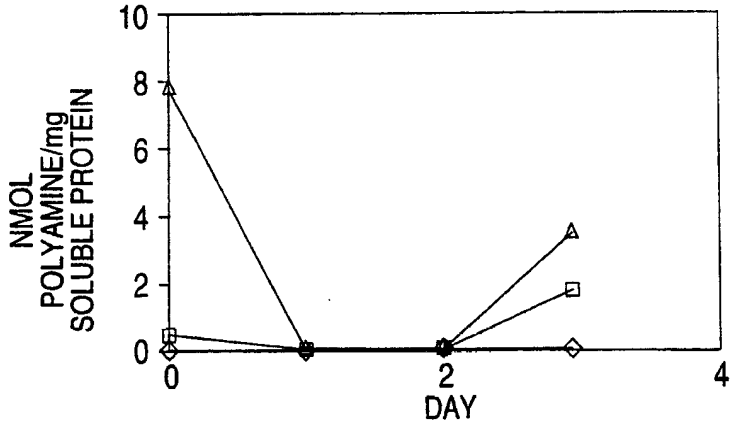

Fig. 8c

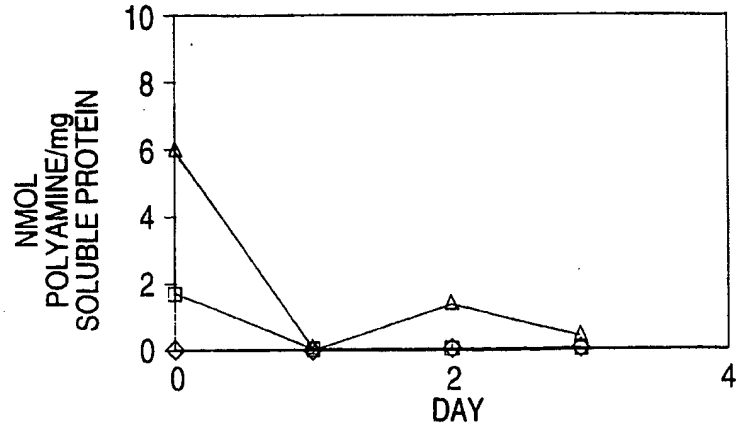

Fig. 8d

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 97/23027

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 5 614 557 A (BEY P. ET AL.) 25 March 1997 see the whole document, especially column 2 lines 36-46 ---	1,4-8
Y	EP 0 357 029 A (MERRELL DOW PHARMACEUTICALS INC.) 7 March 1990 see the whole document ---	1,4-8
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
7 April 1998	13.05.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Gac, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte tional Application No
PCT/US 97/23027

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WAGNER ET AL: "Resolution of the enantiomers of various alpha-substituted ornithine and lysine analogs by high-performence liquid chromatography with chiral eluant and by gas chromatography on Chirasil-Val" ANAL. BIOCHEM., vol. 164, no. 1, July 1987, pages 102-116, XP002061670 see the whole document	1,4-8
Y	THESE DOCT. PHARMACOL. 1990, UNIV. STRASBOURG 1, FR. (INIST-T 74385) SCHMITT-HOFFMANN A. Pages 1-6,26-44,108-117,234-278,360-370 and "table des matières" XP002061673 see page 31 see page 36 see page 39 see page 234 - page 239 see page 364 - page 370	1,4-8
A	KREMMER ET AL.: "Changes in the polyamine and nucleotide metabolism of P388 leukemia cells treated with DL-alpha-difluoromethylornithine in culture" EXP. CELL. BIOL., vol. 56, no. 3, 1988, pages 131-137, XP002061671 see the whole document	1-10
A	BOWLIN ET AL.: "Effect of polyamine depletion in vivo by DL-alpha-difluoromethylornithine on functionally distinct populations of tumoricidal effector cells in normal and tumor-bearing mice" CANCER RES., vol. 46, no. 11, November 1986, pages 5494-5498, XP002061672 see the whole document	1-10

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/ 23027

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 10
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claim 10, referring to a method, cannot be dependent on previous claims 6-9 as mentioned. Correction should be made accordingly.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte ional Application No
PCT/US 97/23027

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5614557 A	25-03-97	AU 522660 B	17-06-82
		AU 3732778 A	03-01-80
		BE 868882 A	03-11-78
		BE 881208 A	16-05-80
		BE 881209 A	16-05-80
		CA 1091661 A	16-12-80
		CH 642055 A	30-03-84
		DE 2828739 A	01-02-79
		FR 2430418 A	01-02-80
		GB 2001960 A,B	14-02-79
		JP 1022259 B	25-04-89
		JP 1538170 C	16-01-90
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		NL 7807453 A,B,	15-01-79
		SE 7807691 A	12-01-79
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		US 4743691 A	10-05-88
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		ES 2053889 T	01-08-94
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		US 5217886 A	08-06-93